CEREBRAL AND SPLANCHNIC OXYGENATION AND NECROTIZING ENTEROCOLITIS IN PRETERM INFANTS

NYNKE T.E. SCHAT

Cover design and layout: Proefschriftmaken.nl || Uitgeverij BOXPress
Printed by: Proefschriftmaken.nl || Uitgeverij BOXPress

ISBN 978-94-6295-207-2
Digitale ISBN 978-94-6295-214-0

I greatly acknowledge the financial support of the Sint Christophorileen tot Oldehove, which I received as descendant of Gerrit van Belcum, founder of this trust.

Additionally, the printing of this thesis was financially supported by:
Rijksuniversiteit Groningen, Universitair Medisch Centrum Groningen, postgraduate school for Behavioural and Cognitive Neurosciences.

rijksuniversiteit groningen

Cerebral and splanchnic oxygenation and necrotizing enterocolitis in preterm infants

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. E. Sterken
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 1 juli 2015 om 14.30 uur

door

Trijntje Eelkje Schat

geboren op 2 februari 1988
te Dongeradeel

Promotor
Prof. dr. A.F. Bos

Copromotores
Dr. E.M.W. Kooi
Dr. J.B.F. Hulscher

Beoordelingscommissie
Prof. dr. E. Heineman
Prof. dr. F. van Bel
Prof. dr. I. Reiss

Paranimfen
Maaike A. Schat
Tineke S. Dijkstra-Taal

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

Trijntje E. Schat

The main goal of this thesis is to investigate whether monitoring cerebral and splanchnic oxygenation by means of near-infrared spectroscopy (NIRS) could be useful in infants who develop necrotizing enterocolitis (NEC). NEC is currently the most common and deadliest gastrointestinal disease of prematurity.[1] The first observations and reports of NEC can be traced back to the beginning of the nineteenth century. Back then, NEC was still considered a rare disease. However, with the founding of the first neonatal intensive care units in the 1960s, the prevalence of NEC increased rapidly.[2] Despite improvements in neonatal care and extensive research regarding the pathophysiology of NEC, we are still unable to predict the onset of NEC, and offer appropriate and timely treatment, to counteract the devastating short- and long-term consequences with which NEC is associated.

NECROTIZING ENTEROCOLITIS

Epidemiology

NEC primarily affects preterm infants; more than 85% of NEC cases occur in infants with a birth weight below 1500 grams or with a gestational age of less than 32 weeks.[1,3,4] In those instances where NEC occurs in term and late preterm infants, an underlying condition is often present that causes intestinal hypoxia and/or hypoperfusion, such as congenital heart disease and intestinal anomalies.[5,6]

The most important factor that determines the prevalence of NEC is the neonatal intensive care unit in which an infant was cared for.[7,8] Large multicenter and population based studies estimated the prevalence of NEC in very low birth weight infants at between 7 and 11%.[8-10]

Pathophysiology

The pathophysiology of NEC remains elusive; there are, however, several factors that are believed to predispose an infant to developing NEC. These factors include, amongst others, intestinal immaturity, enteral feeding, microbial colonization, and an imbalance in intestinal microvascular tone.[1] It goes beyond the scope of this thesis to elaborate on the presumed role of each of these factors; only the role of intestinal perfusion will be further discussed.

In 1969, Lloyd observed an association between severe, sustained asphyxia and gastrointestinal perforation. He was the first to propose that intestinal hypoxia might be one of the principal factors leading to the development of NEC and suggested that redistribution of blood flow to the brain, heart, and kidneys caused a markedly reduced intestinal perfusion ('the diving reflex').[11] It is still believed that intestinal hypoperfusion and hypoxia play a considerable role in the development of NEC. In the neonatal rat model of NEC, which is currently the best accepted model, hypoxia-ischemia is one of the essential factors needed to generate NEC.[12,13] Furthermore, it was found that the intestinal tissue of infants with NEC, whether diagnosed or suspected for NEC but not clinically or radiologically confirmed, was poorly perfused using intravenous fluorescein during laparoscopy.[14] Additionally, a high resistance pattern of flow in the superior mesenteric artery was found in preterm infants with necrotizing enterocolitis, suggesting compromised intestinal blood flow.[15,16] Currently,

the mechanism responsible for intestinal hypoxia is thought to be quite different from the one proposed by Lloyd. It is hypothesized that an imbalance between vasodilatory and vasoconstrictor regulation in favor of vasoconstriction contributes to ischemic injury.[17-19] It remains unknown whether ischemic injury is (one of) the primary inciting factor(s) or is merely a secondary development as a result of intestinal inflammation and mucosal injury.[19]

Clinical presentation and management

Symptoms that indicate the presence of NEC can be of both abdominal and systemic origin, such as abdominal distention, feeding intolerance, apnea, bradycardia, and temperature instability.[1,20] Common laboratory findings are leukocytosis, thrombocytopenia, metabolic acidosis, and increased C-reactive protein levels.[20] Unfortunately, these symptoms and signs are nonspecific and can be found in the presence of a variety of other diagnoses, such as sepsis.[20] The only signs that confirm the presence of NEC are pneumatosis intestinalis and/or portal venous gas on abdominal radiographic examination.[1] These findings, however, might become evident only in advanced disease.

The course of NEC can be uncomplicated in which case the clinical symptoms and radiographic signs resolve gradually over days. However, progression to complicated NEC can be sudden with perforation, peritonitis, sepsis, and death in just a couple of hours. Since it remains impossible to predict the course of NEC, all infants who are suspected of NEC are treated uniformly, consisting of nil per mouth, gastric suctioning, and antibiotics, and by re-evaluation of abdominal symptoms and radiographic signs regularly. When a perforation is suspected or when an infant is clinically deteriorating, surgical treatment is required.

In order to help and guide clinicians in diagnosing and treating NEC, several staging systems have been developed.[21-23] In our neonatal intensive care unit, the modified Bell's staging system is used.[22] This staging system defines three stages, each divided into two subcategories (Table 1).[24] In case of Bell's stage 1, or suspected NEC, preterm infants have nonspecific abdominal and systemic symptoms suggesting NEC, but which cannot be confirmed by abdominal radiographic examination. Often, these infants are eventually diagnosed with diseases other than NEC. Bell's stage 2 is frequently referred to as definite or proven NEC. Pneumatosis intestinalis, portal venous gas, or both are present on imaging findings. Finally, we distinguish Bell's stage 3, or advanced NEC. This stage is characterized by deteriorating vital signs, such as hypotension and combined metabolic and respiratory acidosis. In addition to the radiographic signs described for Bell's stages 1 and 2, pneumoperitoneum ('free air') can also be present. This finding warrants immediate surgical interference.

Although the use of the modified Bell's staging system is widely employed, there are several limitations. Firstly, the stages described do not need to be followed in a sequential manner. Although it occurs rarely, the first sign of NEC can be pneumoperitoneum which classifies an infant directly into Bell's stage 3. Secondly and more importantly, due to the nonspecific symptoms used to define Bell's stage 1, many infants are classified as such and treated accordingly. The majority of these infants, however, are eventually diagnosed differently.

Table 1. Modified Bell's staging criteria for necrotizing enterocolitis.

Stage	Classification	Systemic signs	Intestinal signs	Radiologic signs
1A	Suspected NEC	Temperature instability, apnea, bradycardia, lethargy	Increased pregavage residuals, mild abdominal distention, emesis, guaiac-positive stool	Normal or intestinal dilation, mild ileus
1B	Suspected NEC	Same as above	Bright red blood from rectum	Same as above
2A	Proven NEC – mildly ill	Same as above	Same as above, plus absent bowel sounds, with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
2B	Proven NEC – moderately ill	Same as above, plus mild metabolic acidosis, mild thrombocytopenia	Same as above, plus absent bowel sounds, definite abdominal tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as 2A, plus portal venous gas, with or without ascites
3A	Advanced NEC – severely ill, bowel intact	Same as 2B, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, disseminated intravascular coagulation, and neutropenia	Same as above, plus signs of generalized peritonitis, marked tenderness, and distention of abdomen	Same as 2B, plus definite ascites
3B	Advanced NEC – severely ill, bowel perforated	Same as 3A	Same as 3A	Same as 2B, plus pneumoperitoneum

Modified from J.S. Lee and R.A. Polin.[24] NEC - necrotizing enterocolitis.

Marker of intestinal damage in NEC

A protein that represents the degree of intestinal damage in infants with NEC is intestinal fatty acid-binding protein (I-FABP). I-FABP is a small intracellular protein that plays an important role in the fatty acid metabolism and transport.[25] It is primarily located in epithelium cells of the small bowel, with only trace amounts in the stomach and large intestine.[25] Normally,

I-FABP is present in very small quantities in the circulation.[26] However, levels rapidly increase in the event of compromised cell membrane integrity, such as occurs in acute intestinal ischemia and inflammation, including NEC.[25,27-29] Because I-FABP has a low molecular weight, it passes through the glomerular filter, and can be easily and rapidly detected in the urine.[26] Since the bladder serves as a storage site, I-FABP levels in urine represent the secretion of I-FABP over a longer period of time, whilst I-FABP levels in plasma represent the immediate secretion by enterocytes.[25] It was found that I-FABP levels in both plasma and urine are associated with the development of NEC and its severity.[28-32] However, determining I-FABP levels is not yet sufficiently accurate to be considered diagnostic for NEC.[33]

Outcome and complications

Despite significant progress in the field of neonatology, the short- and long-term consequences of NEC have not improved. Overall, mortality rates range between 9 and 40%, and increase with lower birth weight and severity of NEC.[9,34-36] Other complications include intestinal stricture, abdominal abcess, cholestasis, and short bowel syndrome.[3,37,38] Moreover, it was found that infants who survived NEC, specifically those who were treated surgically, had neurodevelopmental impairments later on.[39-43]

NEAR-INFRARED SPECTROSCOPY

Since an impaired intestinal perfusion seems to play an essential role in the development of NEC, being able to detect this altered perfusion may give the clinician an early warning about the onset and progression of this disease. It was proposed that NIRS monitoring could be helpful in identifying infants with impaired bowel perfusion.[44]

Technique

In 1977, Jöbsis introduced NIRS as a method to non-invasively monitor the oxygen saturation of tissue.[45] A couple of years later the first report appeared that demonstrated that NIRS could be used at the bedside to monitor cerebral oxygen saturation in sick preterm infants.[46] NIRS is based on the fact that light in the near-infrared range (wavelengths between 700 and 1000 nm) can be effectively transmitted through biological tissue over longer distances.[45] Within these wavelengths, the majority of near-infrared light will be absorbed by oxygenated and deoxygenated hemoglobin, each of which have a distinct absorption spectrum.[47] The remaining light will be either reflected or scattered. NIRS measures the spectral absorption for oxygenated and deoxygenated hemoglobin separately, and then calculates the ratio of oxygenated hemoglobin to total hemoglobin. This measurement represents the oxygen uptake in tissue and is referred to as regional tissue oxygen saturation (rSO_2). Approximately 75 to 80% of this value forms a representation of the saturation of venous blood, 5% forms the capillary compartment and the remaining forms arterial blood.[48,49]

For the purpose of this thesis, we used the INVOS 5100C spectrometer (Covidien, Mansfield, MA, USA) with neonatal SomaSensors (Covidien). The SomaSensor has one light emitting

diode that emits two wavelengths into underlying tissue, i.e. 730 and 810 nm. A shallow and a deep detector, at 30 and 40 mm distance from the emitter respectively, receive the light as a function of wavelength. The shallow detector provides information about surface tissue oxygen saturation and the deep detector information about the oxygen saturation of deeper tissues. The rSO_2 is calculated by subtracting the oxygen saturation of the surface path from the deeper path and represents the venous weighted oxygen saturation of tissues at a depth of approximately 20 mm.[48,50]

When the transcutaneous arterial oxygen saturation (SpO_2) is measured simultaneously, the fractional tissue oxygen extraction (FTOE) can be calculated: FTOE = (SpO_2 - rSO_2)/ SpO_2.[51,52] It is thought that FTOE reflects the balance between tissue oxygen supply and tissue oxygen consumption and might therefore be an early indicator of impaired tissue perfusion.[52] High FTOE values can indicate two possibilities: (1) increased oxygen extraction due to increased metabolism at the tissue level, or (2) increased oxygen extraction due to decreased blood flow to the tissue that is being measured.

NIRS and NEC

Studies investigating intestinal perfusion with NIRS in both animals and humans showed promising results. Fortune and colleagues were the first to find a difference in NIRS measurements between infants with and infants without bowel ischemia.[44] Instead of measuring intestinal tissue alone, they also measured the oxygen saturation of cerebral tissue. They used the cerebral rSO_2 value as a reference to calculate the cerebro-splanchnic oxygenation ratio (CSOR) and found that a CSOR < 0.75 predicted the development of bowel ischemia with a sensitivity of 0.90 and a specificity of 0.96. Additionally, several reports were published which showed increasing splanchnic rSO_2 values in infants who were recovering from NEC, demonstrated by improved clinical and radiographic signs and symptoms.[53,54] Finally, it was found that intestinal rSO_2 values were low with little variability in two infants, several days prior to NEC development.[55]

It was also suggested that infants in whom NEC develops already have an altered intestinal perfusion from birth onwards.[56,57] A study using a piglet model showed that abdominal NIRS readings were indeed lower on the first day after birth in piglets that developed NEC compared to those that did not.[56] This finding was recently confirmed in a large study performed in preterm infants with a gestational age of less than 32 weeks and a birth weight below 1500 grams.[57]

However, there are some concerns regarding monitoring rSO_2 in the intestinal region.

Since the bowel is a hollow organ, enteric contents, such as meconium and air, may be measured in stead of or concomitantly with intestinal tissue.[58,59] It was found that meconium can alter the reflected signal considerably.[60] Furthermore, peristalsis and movements of the gut within the abdominal cavity may alter the tissue that is being sampled during static sensor placement. For these reasons, it was suggested that the liver region could be a better site for monitoring rSO_2 of intestinal tissue.[58] The liver not only receives oxygenated blood

from the hepatic artery, but deoxygenated blood, that already passed the intestine, from the portal vein as well. Moreover, the liver is a solid and non-moving organ.
Finally, since impaired splanchnic perfusion might be the result of a compromised systemic circulation, it would also be interesting to measure cerebral oxygenation values.

In this thesis we define the oxygenation of intestinal tissue as splanchnic oxygenation. We measured this oxygenation using NIRS at two abdominal locations: in the infraumbilical region on the central abdomen (intestinal or infraumbilical oxygenation), and the liver region, located at the right upper quadrant just below the costal margin (liver oxygenation).

OUTLINE AND AIMS OF THE THESIS

Our main aim was to investigate whether monitoring cerebral, liver, and intestinal oxygenation could be useful in infants who develop NEC. To this end, we first investigated the feasibility and validity of monitoring cerebral and splanchnic oxygenation by NIRS. Second, we investigated whether these NIRS measurements can be used for three purposes, i.e. (1) identifying infants who go on to develop NEC in infants with a high risk of developing NEC, (2) identifying infants with definite NEC in infants with abdominal signs and symptoms, and (3) identifying infants with complicated NEC in infants with established NEC. Complicated NEC was defined as the infant developing a bowel perforation requiring surgery (Bell's stage 3B), or death.

The specific research questions were (chapters which focus on each question are indicated in brackets):

(1) Is it feasible to study splanchnic oxygenation simultaneously in two abdominal regions in infants with suspected and definite NEC? Can liver and infraumbilical oxygen saturation values substitute each other for the purpose of assessing splanchnic oxygenation? (Chapter 2)
(2) Can cerebral and splanchnic FTOE values be used as markers for intestinal damage in infants with NEC? (Chapter 3)
(3) Do preterm infants with NEC show impaired cerebrovascular autoregulation more often than infants without NEC? (Chapter 4)
(4) Can we differentiate high-risk infants who develop NEC from those who do not by monitoring cerebral and intestinal oxygenation as early as in the first days after birth? (Chapter 5)
(5) Can we, in an early stage of the disease, differentiate infants with definite NEC from infants with suspected NEC, and infants with complicated NEC from infants with uncomplicated NEC by monitoring cerebral, liver, and intestinal oxygenation? (Chapter 6)

REFERENCES

1. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011;364(3):255-264.
2. Obladen M. Necrotizing enterocolitis – 150 years of fruitless search for the cause. *Neonatology*. 2009;96(4):203-210.
3. Sharma R, Hudak ML, Tepas JJ,3rd, et al. Impact of gestational age on the clinical presentation and surgical outcome of necrotizing enterocolitis. *J Perinatol*. 2006;26(6):342-347.
4. Thompson AM, Bizzarro MJ. Necrotizing enterocolitis in newborns: Pathogenesis, prevention and management. *Drugs*. 2008;68(9):1227-1238.
5. Martinez-Tallo E, Claure N, Bancalari E. Necrotizing enterocolitis in full-term or near-term infants: Risk factors. *Biol Neonate*. 1997;71(5):292-298.
6. Raboei EH. Necrotizing enterocolitis in full-term neonates: Is it aganglionosis? *Eur J Pediatr Surg*. 2009;19(2):101-104.
7. Uauy RD, Fanaroff AA, Korones SB, Phillips EA, Phillips JB, Wright LL. Necrotizing enterocolitis in very low birth weight infants: Biodemographic and clinical correlates. National institute of child health and human development neonatal research network. *J Pediatr*. 1991;119(4):630-638.
8. Guillet R, Stoll BJ, Cotten CM, et al. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics*. 2006;117(2):e137-42.
9. Fitzgibbons SC, Ching Y, Yu D, et al. Mortality of necrotizing enterocolitis expressed by birth weight categories. *J Pediatr Surg*. 2009;44(6):1072-5; discussion 1075-6.
10. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD neonatal research network. *Pediatrics*. 2010;126(3):443-456.
11. Lloyd JR. The etiology of gastrointestinal perforations in the newborn. *J Pediatr Surg*. 1969;4(1):77-84.
12. Barlow B, Santulli TV. Importance of multiple episodes of hypoxia or cold stress on the development of enterocolitis in an animal model. *Surgery*. 1975;77(5):687-690.
13. Caplan MS, Hedlund E, Adler L, Hsueh W. Role of asphyxia and feeding in a neonatal rat model of necrotizing enterocolitis. *Pediatr Pathol*. 1994;14(6):1017-1028.
14. Numanoglu A, Millar AJ. Necrotizing enterocolitis: Early conventional and fluorescein laparoscopic assessment. *J Pediatr Surg*. 2011;46(2):348-351.
15. Kempley ST, Gamsu HR. Superior mesenteric artery blood flow velocity in necrotising enterocolitis. *Arch Dis Child*. 1992;67(7 Spec No):793-796.
16. Deeg KH, Rupprecht T, Schmid E. Doppler sonographic detection of increased flow velocities in the celiac trunk and superior mesenteric artery in infants with necrotizing enterocolitis. *Pediatr Radiol*. 1993;23(8):578-582.
17. Nowicki PT. Ischemia and necrotizing enterocolitis: Where, when, and how. *Semin Pediatr Surg*. 2005;14(3):152-158.
18. Nankervis CA, Giannone PJ, Reber KM. The neonatal intestinal vasculature: Contributing factors to necrotizing enterocolitis. *Semin Perinatol*. 2008;32(2):83-91.
19. Watkins DJ, Besner GE. The role of the intestinal microcirculation in necrotizing enterocolitis. *Semin Pediatr Surg*. 2013;22(2):83-87.
20. Dominguez KM, Moss RL. Necrotizing enterocolitis. *Clin Perinatol*. 2012;39(2):387-401.
21. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg*. 1978;187(1):1-7.
22. Walsh MC, Kliegman RM. Necrotizing enterocolitis: Treatment based on staging criteria. *Pediatr Clin North Am*. 1986;33(1):179-201.

23. Gordon PV, Swanson JR, Attridge JT, Clark R. Emerging trends in acquired neonatal intestinal disease: Is it time to abandon Bell's criteria? *J Perinatol*. 2007;27(11):661-671.
24. Lee JS, Polin RA. Treatment and prevention of necrotizing enterocolitis. *Semin Neonatol*. 2003;8(6):449-459.
25. Lieberman JM, Sacchettini J, Marks C, Marks WH. Human intestinal fatty acid binding protein: Report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery*. 1997;121(3):335-342.
26. Derikx JP, Evennett NJ, Degraeuwe PL, et al. Urine based detection of intestinal mucosal cell damage in neonates with suspected necrotising enterocolitis. *Gut*. 2007;56(10):1473-1475.
27. Kanda T, Fujii H, Tani T, et al. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology*. 1996;110(2):339-343.
28. Edelson MB, Sonnino RE, Bagwell CE, Lieberman JM, Marks WH, Rozycki HJ. Plasma intestinal fatty acid binding protein in neonates with necrotizing enterocolitis: A pilot study. *J Pediatr Surg*. 1999;34(10):1453-1457.
29. Guthmann F, Borchers T, Wolfrum C, Wustrack T, Bartholomaus S, Spener F. Plasma concentration of intestinal- and liver-FABP in neonates suffering from necrotizing enterocolitis and in healthy preterm neonates. *Mol Cell Biochem*. 2002;239(1-2):227-234.
30. Evennett NJ, Hall NJ, Pierro A, Eaton S. Urinary intestinal fatty acid-binding protein concentration predicts extent of disease in necrotizing enterocolitis. *J Pediatr Surg*. 2010;45(4):735-740.
31. Aydemir C, Dilli D, Oguz SS, et al. Serum intestinal fatty acid binding protein level for early diagnosis and prediction of severity of necrotizing enterocolitis. *Early Hum Dev*. 2011;87(10):659-661.
32. Heida FH, Hulscher JBF, Schurink M, et al. Intestinal fatty acid-binding protein levels in necrotizing enterocolitis correlate with extent of necrotic bowel: results from a multicenter study. *J Pediatr Surg.* 2014.
33. Evennett N, Alexander N, Petrov M, Pierro A, Eaton S. A systematic review of serologic tests in the diagnosis of necrotizing enterocolitis. *J Pediatr Surg*. 2009;44(11):2192-2201.
34. Guthrie SO, Gordon PV, Thomas V, Thorp JA, Peabody J, Clark RH. Necrotizing enterocolitis among neonates in the United States. *J Perinatol*. 2003;23(4):278-285.
35. Holman RC, Stoll BJ, Curns AT, Yorita KL, Steiner CA, Schonberger LB. Necrotising enterocolitis hospitalisations among neonates in the United States. *Paediatr Perinat Epidemiol*. 2006;20(6):498-506.
36. Berrington JE, Hearn RI, Bythell M, Wright C, Embleton ND. Deaths in preterm infants: Changing pathology over 2 decades. *J Pediatr*. 2012;160(1):49-53.e1.
37. Guner YS, Friedlich P, Wee CP, Dorey F, Camerini V, Upperman JS. State-based analysis of necrotizing enterocolitis outcomes. *J Surg Res*. 2009;157(1):21-29.
38. Abdullah F, Zhang Y, Camp M, et al. Necrotizing enterocolitis in 20,822 infants: Analysis of medical and surgical treatments. *Clin Pediatr (Phila)*. 2010;49(2):166-171.
39. Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA*. 2004;292(19):2357-2365.
40. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. *Arch Dis Child Fetal Neonatal Ed*. 2007;92(3):F193-8.
41. Schulzke SM, Deshpande GC, Patole SK. Neurodevelopmental outcomes of very low-birth-weight infants with necrotizing enterocolitis: A systematic review of observational studies. *Arch Pediatr Adolesc Med*. 2007;161(6):583-590.
42. Martin CR, Dammann O, Allred EN, et al. Neurodevelopment of extremely preterm infants who had necrotizing enterocolitis with or without late bacteremia. *J Pediatr*. 2010;157(5):751-6.e1.

43. Roze E, Ta BD, van der Ree MH, et al. Functional impairments at school age of children with necrotizing enterocolitis or spontaneous intestinal perforation. *Pediatr Res*. 2011;70(6):619-625.
44. Fortune PM, Wagstaff M, Petros AJ. Cerebro-splanchnic oxygenation ratio (CSOR) using near infrared spectroscopy may be able to predict splanchnic ischaemia in neonates. *Intensive Care Med*. 2001;27(8):1401-1407.
45. Jobsis FF. Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science*. 1977;198(4323):1264-1267.
46. Brazy JE, Lewis DV, Mitnick MH, Jobsis vander Vliet FF. Noninvasive monitoring of cerebral oxygenation in preterm infants: Preliminary observations. *Pediatrics*. 1985;75(2):217-225.
47. Wyatt JS, Cope M, Delpy DT, Wray S, Reynolds EO. Quantification of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet*. 1986;2(8515):1063-1066.
48. Watzman HM, Kurth CD, Montenegro LM, Rome J, Steven JM, Nicolson SC. Arterial and venous contributions to near-infrared cerebral oximetry. *Anesthesiology*. 2000;93(4):947-953.
49. Wong FY, Alexiou T, Samarasinghe T, Brodecky V, Walker AM. Cerebral arterial and venous contributions to tissue oxygenation index measured using spatially resolved spectroscopy in newborn lambs. *Anesthesiology*. 2010;113(6):1385-1391.
50. Mudra R, Nadler A, Keller E, Niederer P. Analysis of near-infrared spectroscopy and indocyanine green dye dilution with monte carlo simulation of light propagation in the adult brain. *J Biomed Opt*. 2006;11(4):044009.
51. Kissack CM, Garr R, Wardle SP, Weindling AM. Cerebral fractional oxygen extraction is inversely correlated with oxygen delivery in the sick, newborn, preterm infant. *J Cereb Blood Flow Metab*. 2005;25(5):545-553.
52. Naulaers G, Meyns B, Miserez M, et al. Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation. A validation study in piglets. *Neonatology*. 2007;92(2):120-126.
53. Stapleton GE, Eble BK, Dickerson HA, Andropoulos DB, Chang AC. Mesenteric oxygen desaturation in an infant with congenital heart disease and necrotizing enterocolitis. *Tex Heart Inst J*. 2007;34(4):442-444.
54. Zabaneh RN, Cleary JP, Lieber CA. Mesentric oxygen saturations in premature twins with and without necrotizing enterocolitis. *Pediatr Crit Care Med*. 2011;12(6):e404-6.
55. Cortez J, Gupta M, Amaram A, Pizzino J, Sawhney M, Sood BG. Noninvasive evaluation of splanchnic tissue oxygenation using near-infrared spectroscopy in preterm neonates. *J Matern Fetal Neonatal Med*. 2011;24(4):574-582.
56. Gay AN, Lazar DA, Stoll B, et al. Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. *J Pediatr Surg*. 2011;46(6):1034-1040.
57. Patel AK, Lazar DA, Burrin DG, et al. Abdominal near-infrared spectroscopy measurements are lower in preterm infants at risk for necrotizing enterocolitis. *Pediatr Crit Care Med*. 2014;15(8):735-741.
58. Teller J, Wolf M, Keel M, Bucher HU, Fanconi S, Baenziger O. Can near infrared spectroscopy of the liver monitor tissue oxygenation? *Eur J Pediatr*. 2000;159(7):549.
59. McNeill S, Gatenby JC, McElroy S, Engelhardt B. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. *J Perinatol*. 2011;31(1):51-57.

60. Thompson A, Benni P, Seyhan S, Ehrenkranz R. Meconium and transitional stools may cause interference with near-infrared spectroscopy measurements of intestinal oxygen saturation in preterm infants. *Adv Exp Med Biol*. 2013;765:287-292.

CHAPTER 2

ABDOMINAL NEAR-INFRARED SPECTROSCOPY IN PRETERM INFANTS: A COMPARISON OF SPLANCHNIC OXYGEN SATURATION MEASUREMENTS AT TWO ABDOMINAL LOCATIONS

Trijntje E. Schat, Michelle E. van der Laan, Maarten Schurink, Jan B.F. Hulscher, Christian V. Hulzebos, Arend F. Bos, Elisabeth M.W. Kooi

Early Human Development 2014;90:371-375

ABSTRACT

Background: Splanchnic tissue oxygenation monitoring has been performed at both the liver and the infraumbilical regions. It is unknown whether these measurements could be substituted one for the other when interpreting splanchnic oxygenation since they have not been measured simultaneously before.

Aims: To evaluate the feasibility and safety of liver and infraumbilical near-infrared spectroscopy (NIRS) monitoring in preterm infants with suspected necrotizing enterocolitis (NEC) and to assess the correlation and agreement between NIRS measurements performed simultaneously at the two abdominal locations.

Study design and subjects: This study was part of a prospective observational cohort study. Preterm infants who were suspected of NEC or who had been diagnosed with NEC were included.

Outcome measures: Liver oxygen saturation and infraumbilical oxygen saturation were monitored simultaneously and continuously for 48 hours by NIRS.

Results: NIRS monitoring was performed in 20 out of 24 infants for the entire 48-hour study period. No adverse effects were observed. Values of liver and infraumbilical oxygen saturation correlated weakly (Spearman's rho = 0.244, $P < .001$). On the Bland-Altman plot liver oxygen saturation was higher than infraumbilical oxygen saturation (mean difference 6.6%, SD 22.5%).

Conclusions: Using NIRS as method for monitoring oxygen saturation simultaneously in both the liver and infraumbilical regions is safe and feasible. Additionally, we demonstrated that values of liver and infraumbilical oxygen saturation cannot be randomly substituted one for the other for the purpose of assessing splanchnic oxygenation.

INTRODUCTION

Near-infrared spectroscopy (NIRS) is a non-invasive tool that can be used to continuously measure the oxygen saturation of underlying tissue.[1] It is being used increasingly to investigate splanchnic oxygen saturation. Various locations, including the liver and infraumbilical regions, have been selected for placing the NIRS sensors for this purpose.[2-10] Although this new technique seems promising for assessing splanchnic oxygen saturation, location-specific characteristics have been identified that may interfere with the reliability of the measurements. The infraumbilical region covers non-solid and moving tissue. Movements of the intestines within the abdominal cavity as well as peristaltic movements may alter the reflected signal despite static sensor placement.[11] The liver, a solid and non-moving organ, relies on the portal vein for approximately 75% of its blood supply and on the hepatic artery for the remaining 25%. Due to the hepatic arterial buffer response, no direct linear relationship exists between the contributions of the two vessels to the hepatic blood supply, thus limiting the potential use of NIRS to monitor splanchnic oxygenation.[12] To date, it remains unclear whether liver and infraumbilical oxygen saturation measurements obtained by NIRS can be substituted one for the other for interpreting splanchnic oxygenation, since splanchnic oxygen saturation in the liver and infraumbilical regions have not been monitored simultaneously before. Additionally, it is difficult to compare the liver and infraumbilical oxygen saturation values reported in the literature due to discrepancies between study groups and study methods.

The primary aim of this study was to evaluate the feasibility and safety of monitoring splanchnic oxygen saturation in the liver and infraumbilical regions simultaneously by NIRS in preterm infants with suspected necrotizing enterocolitis (NEC). Our secondary aim was to compare the liver measurements with the infraumbilical measurements and to assess the correlation and agreement between the oxygen saturation values obtained at the two abdominal locations.

METHODS

Ethical statement

This study was part of a prospective observational cohort study registered with the Dutch Trial Registry under number NTR3239. The study was approved by the ethical review board of University Medical Center Groningen. Written informed parental consent was obtained in all cases.

Patients and procedures

We included preterm infants admitted to the neonatal intensive care unit of University Medical Center Groningen between October 2010 and March 2012, who were suspected of NEC or who had been diagnosed with NEC. Suspected NEC is defined as Bell's stage 1, in which case only non-specific symptoms of abdominal disease, such as gastric retention,

abdominal distension, and mild ileus, are present.[13,14] Infants with abdominal wall defects were excluded. Monitoring splanchnic oxygen saturation by NIRS commenced as soon as possible after suspected or diagnosed NEC and was continued for 48 hours.

Near-infrared spectroscopy

We used the INVOS 5100C near-infrared spectrometer (Covidien, Mansfield, MA, USA) in combination with the neonatal SomaSensors (Covidien) to measure splanchnic oxygen saturation continuously and simultaneously in both the liver and infraumbilical regions. Near-infrared light is emitted using two wavelengths (730 and 810 nm). By measuring the quantity of reflected light as a function of wavelength, the spectral absorption of the underlying tissue can be calculated. Since oxygenated and deoxygenated hemoglobin have different absorption spectra, NIRS can differentiate between the two. The ratio of oxygenated hemoglobin to total hemoglobin reflects the regional tissue oxygen saturation (rSO_2). The SomaSensor has a shallow and deep detector; on 3 and 4 cm distance from the near-infrared optode respectively. By subtracting the measurement of the shallow detector from the deep detector, oxygenation values of the deep detector, which reflect the tissue beneath the skin, are calculated. The depth of the signal is estimated to be around 15 to 20 mm.[15]

For this study, we placed the neonatal SomaSensors just below the right costal arch to measure liver oxygen saturation ($r_{liv}SO_2$) and just below the umbilicus on the central abdomen to measure intestinal oxygen saturation ($r_{int}SO_2$). The SomaSensors were held in place by elastic bandaging and were removed only during moments of routine nursing care, clinical assessment, and radiographic examination; afterwards they were replaced onto the same location. There was no overlap between the sensors at any time. $R_{liv}SO_2$ and $r_{int}SO_2$ were measured every 6 seconds for 48 hours. The measurements were saved on the INVOS 5100C near-infrared spectrometer and were downloaded at the end of the study and stored off-line for future analysis. Afterward we only removed data obtained during documented incorrect sensor placement.

Clinical variables

We prospectively collected neonatal characteristics including gestational age, postnatal age at first NIRS measurement, birth weight, and gender. We documented the following characteristics as well: respiratory support at the time of NEC suspicion/diagnosis, mean systemic blood pressure in the first hour after start of NIRS monitoring, patency of the ductus arteriosus (PDA) from 48 hours before NEC suspicion/diagnosis until the first 48 hours after NEC suspicion/diagnosis or until surgery took place, whichever came first, whether or not the PDA was hemodynamically significant, the first lactate value, and need for fluid resuscitation and inotropes for circulatory support from 1 hour before NEC suspicion/diagnosis until 48 hours after NEC suspicion/diagnosis, or until surgery took place, whichever came first.

Hemodynamically significant PDA was defined as a diastolic forward flow in the branches of the pulmonary artery, a diastolic backflow in the descending aorta, and a left ventricular end diastolic diameter > p 95.

Statistical analysis

We used medians (range) to describe sample characteristics. To determine and compare the courses of $r_{liv}SO_2$ and $r_{int}SO_2$, we calculated mean 1-hour and mean 12-hour values of 5-minute measurements of $r_{liv}SO_2$ and $r_{int}SO_2$ during the 48-hour study period. The 5-minute measurement is based on one oxygen saturation value obtained during these 5 minutes. The differences between the simultaneously obtained 12-hour mean values of $r_{liv}SO_2$ and $r_{int}SO_2$ were analyzed using the Wilcoxon signed rank test.

To determine the variability of the measurements, we calculated each infant's daily intraindividual variability, defined as the daily percentage of time that 1-hour mean $r_{liv}SO_2$ or $r_{int}SO_2$ values were 15% or more below or above the infant's daily mean.[16]

To compare the two abdominal locations at which we monitored splanchnic oxygen saturation, we determined the correlation coefficient of the mean 1-hour period values of $r_{liv}SO_2$ and $r_{int}SO_2$, using the Spearman rank test. Furthermore, to determine if the direction of change in oxygen saturation was comparable between $r_{liv}SO_2$ and $r_{int}SO_2$ values, we calculated the differences between consecutive 1-hour measurements for $r_{liv}SO_2$ and $r_{int}SO_2$ values independently, leading to 47 delta values per child. We analyzed the correlation between these delta $r_{liv}SO_2$ and $r_{int}SO_2$ values using the Spearman rank test. Finally, we constructed a Bland-Altman plot to assess the agreement between the measurements of the two locations.

Since our primary aim was to assess the feasibility and safety of monitoring in both the liver and infraumbilical regions and to assess the correlation and agreement between oxygen saturation values measured at the two abdominal locations, we did not analyze the correlation between NIRS measurements and type of treatment and/or patient outcome.

We used the Statistical Package for the Social Sciences (IBM SPSS Statistics 22, IBM Corp., Armonk, New York, USA) for all statistical analyses. Statistical significance was defined as $P < .05$.

RESULTS

Patient characteristics

We included 24 infants with a median gestational age of 28.4 weeks (range, 25.0-35.9), a median birth weight of 1279 grams (range, 570-2400), and a median postnatal age at the first measurement of 9 days (range, 3-41). The patient characteristics are presented in Table 1.

Table 1. Patient characteristics.

	N = 24
Gestational age (weeks)	28.4 (25.0-35.9)
Birth weight (grams)	1279 (570-2400)
Male	14 (58)
Postnatal age at first $r_{liv}SO_2$ and $r_{int}SO_2$ measurement (days)	9 (3-41)
Respiratory support	
- None/lowflow	9 (38)
- CPAP	4 (17)
- SiPAP/NIMV	3 (12)
- SIMV/SIPPV	7 (29)
- HFOV	1 (4)
PDA	
- Yes	7 (29)
Hemodynamically significant	3 (43)
Mean systemic blood pressure in the first hour after NEC onset (mmHg) (n = 11)	35 (19-66)
Lactate (mmol/L) (n = 14)	2.1 (1.0-5.5)
Circulatory support	
- Fluid resuscitation	13 (54)
- Inotropes	4 (17)

Data are shown as either median (range) or as n (percentage). CPAP - continuous positive airway pressure; HFOV - high-frequency oscillatory ventilation; NIMV - nasal intermittent mandatory ventilation; PDA - patent ductus arteriosus; $r_{liv}SO_2$ - liver tissue oxygen saturation; $r_{int}SO_2$ - infraumbilical tissue oxygen saturation; SIMV - synchronized intermittent mandatory ventilation; SiPAP - synchronised intermittent positive airway pressure; SIPPV - synchronous positive pressure ventilation.

NIRS monitoring

NIRS monitoring was started within 48 hours after suspected or diagnosed NEC and was continued for 48 hours in twenty infants. In one infant, data were partially lost due to technical problems. In three other infants, NIRS monitoring was stopped after a median of 4 hours (range, 3-11) due to progressive circulatory failure leading to the death of one infant an hour later and due to abdominal surgery for NEC in the other two infants. Three infants were not monitored in the liver region due to shortage of equipment. Two infants were not monitored in the infraumbilical region. In one infant this was because of the inability to place the SomaSensor due to the presence of an umbilical venous catheter, and because of shortage of equipment in the other. In none of the infants did we observe adverse skin effects due to sensor placement during the 48-hour study period. Routine care was not hindered by the sensors either.

We were able to calculate mean $r_{liv}SO_2$ values for 817 (71%) and mean $r_{int}SO_2$ values for 773 (67%) 1-hour periods out of a possible 1152 (48 hours × 24 infants). After excluding the data of the infants who had been monitored at only one location due to shortage of equipment

(n = 4), simultaneously acquired measurements were available for 647 (67%) 1-hour periods out of the possible 960 (48 hours × 20 infants).

Course of $r_{liv}SO_2$ and $r_{int}SO_2$

Figure 1 illustrates the 48-hour course of $r_{liv}SO_2$ and $r_{int}SO_2$ measurements. Oxygen saturation values measured by NIRS in the region of the liver were not significantly different from oxygen saturation values obtained in the infraumbilical region. There was a tendency, however, for higher $r_{liv}SO_2$ values between 12 and 36 hours compared to $r_{int}SO_2$ values (Table 2). The percentage of intraindividual variability of both $r_{liv}SO_2$ and $r_{int}SO_2$ values for each day is presented in Table 3.

Table 2. Comparison of median liver and infraumbilical oxygen saturation values during the 48-hour study period.

Hours	$r_{liv}SO_2$		$r_{int}SO_2$		*P* value
	Median	Range	Median	Range	
0 - 12	62%	22% - 90%	51%	15% - 83%	.212
12 - 24	62%	15% - 86%	56%	22% - 72%	.070
24 - 36	60%	16% - 92%	49%	18% - 86%	.068
36 - 48	51%	25% - 86%	49%	25% - 76%	.408

$r_{liv}SO_2$ - liver oxygen saturation; $r_{int}SO_2$ - infraumbilical oxygen saturation.

Table 3. Median percentage of time 1-hour mean $r_{liv}SO_2$ and $r_{int}SO_2$ measured 15% below or above the daily mean.

Hours	Variability $r_{liv}SO_2$ (%)	Variability $r_{int}SO_2$ (%)
0 - 24	13 (0-33)	13 (0-50)
24 - 48	14 (0-78)	13.5 (0-50)

Data are shown as median (range).

$r_{liv}SO_2$ - liver oxygen saturation; $r_{int}SO_2$ - infraumbilical tissue oxygen saturation.

Correlation and agreement between $r_{liv}SO_2$ and $r_{int}SO_2$

We determined the correlation between the $r_{liv}SO_2$ and $r_{int}SO_2$ values obtained simultaneously during the entire 48-hour study period and found a Spearman's rho of 0.244, $P < .001$. The values representing the change in hourly means of $r_{liv}SO_2$ and $r_{int}SO_2$ were not significantly correlated with each other, Spearman's rho 0.062, $P = .131$. The Bland-Altman plot revealed a mean difference in oxygen saturation between $r_{int}SO_2$ and $r_{liv}SO_2$ of 6.6% (SD 22.5%), with $r_{liv}SO_2$ being the highest (Figure 2).

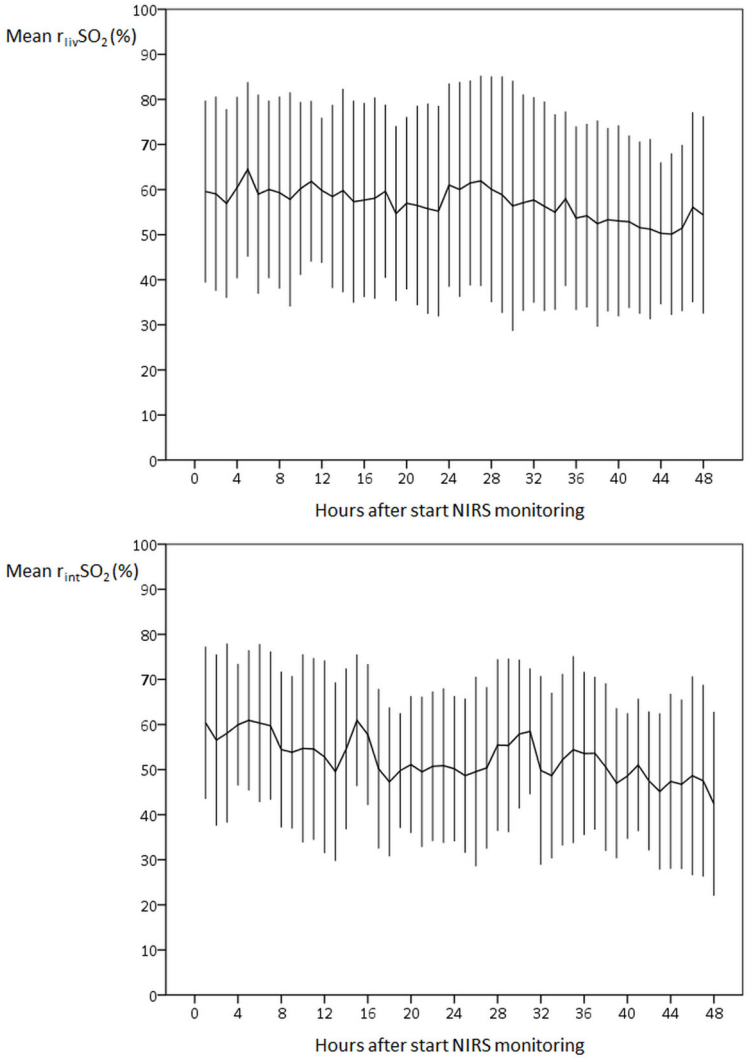

Figure 1. The course of mean liver and infraumbilical oxygen saturation measurements in preterm infants during the first 48 hours after diagnosis of suspected NEC. Error bars represent ± 1 SD.

DISCUSSION

With this study we demonstrated that it is safe and feasible most of the time to use NIRS as method for monitoring tissue oxygen saturation simultaneously at the liver and infraumbilical region. Furthermore we demonstrated that $r_{liv}SO_2$ and $r_{int}SO_2$ values cannot be randomly substituted one for the other for the purpose of assessing splanchnic oxygenation in this population.

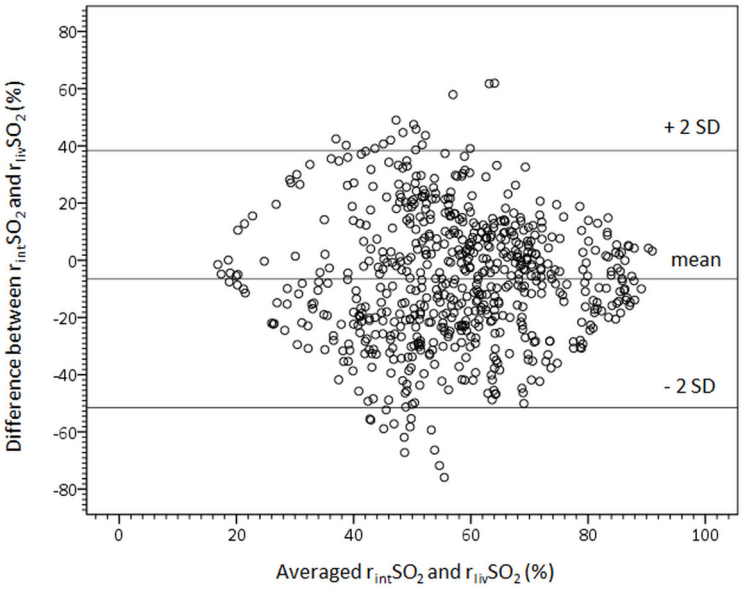

Figure 2. Bland-Altman plot: $r_{int}SO_2$ versus $r_{liv}SO_2$.

NIRS is a non-invasive bedside tool that can be used to monitor tissue oxygenation continuously and as such it seems to be a promising technique, particularly in vulnerable preterm infants. We demonstrated that in preterm infants with suspected NEC it is feasible, for most infants and most of the time, to measure tissue oxygen saturation simultaneously at two abdominal locations. We did not observe any adverse skin effects nor was routine care hampered. Other studies did report adverse skin effects in a few cases.[7,16] A possible explanation for this difference may be the considerably longer monitoring times in these studies of 14 up to 21 days after birth, compared to the 48-hour period in our study.

We were unable to monitor $r_{liv}SO_2$ and $r_{int}SO_2$ simultaneously in five infants. In four infants this was due to shortage of equipment. In one infant, $r_{int}SO_2$ monitoring was not performed due to the lack of space for the sensor because of an umbilical venous catheter taped to the infraumbilical skin, in accordance with local protocol. Theoretically, this situation could be easily overcome. Most preterm infants, however, need intensive, routine care during which clinical procedures and devices might inhibit adequate sensor placement. Nevertheless, our data showed that rarely adequate sensor placement was not possible at all. We were able to monitor liver and infraumbilical oxygen saturation simultaneously 67% of the time.

Because we measured tissue oxygen saturation simultaneously in the liver and infraumbilical regions, we were able to compare the courses of $r_{liv}SO_2$ and $r_{int}SO_2$ values during the study period. The $r_{liv}SO_2$ values (median, 51-62%) were not significantly different from the $r_{int}SO_2$ values (median, 49-56%). There was, however, a tendency for higher oxygen saturation values in the region of the liver compared to the saturation values obtained in the infraumbilical region. There may be several explanations for this finding. Firstly, this tendency could

be attributable to lower oxygen consumption by the liver compared to the intestine in conjunction with the unique portal and arterial hepatic blood supply. A second explanation might be the presence of air, meconium, and/or bilirubin in the preterm intestinal tract. Finally, the underlying intestinal pathophysiological condition might also have played a role. Further studies are needed to delineate the differences in baseline values between $r_{liv}SO_2$ and $r_{int}SO_2$ values and the role of NEC in influencing these oxygen saturation values.

We observed a high variability in the $r_{liv}SO_2$ (13-14%) and $r_{int}SO_2$ values (13-13.5%). Our variability measurements were consistent with those previously reported for the infraumbilical region.[16] McNeill *et al*. speculated that these fluctuations might result from saturation differences between enteral mucosa, smooth muscle, and/or enteric contents in a hollow, moving organ[16] whereas the liver is a solid, non-moving organ comparable to renal tissue. Since renal oxygen saturation values showed less variability,[16] one would also expect $r_{liv}SO_2$ values to be less variable. However, we also found a high variability in the $r_{liv}SO_2$ values. A possible explanation could be that the liver oxygen saturation values reflected the highly variable infraumbilical oxygenation values, for the blood supply to the liver consist for a large part of this splanchnic perfusion. It is also possible that besides the liver, intestinal tissue was measured at the right costal margin. We placed the SomaSensor where we anatomically would expect the liver to be located in these infants, which we did not confirm by ultrasonography. Therefore, we do not know for sure whether we solely measured liver tissue. This is, however, generally the case in a clinical setting.

We found a weak correlation between liver and infraumbilical oxygen saturation values, a lack of correlation between values representing the change in hourly means of both measurements, and the Bland-Altman plot revealed relatively large discrepancies between the $r_{liv}SO_2$ and $r_{int}SO_2$ values. These findings suggest that absolute values as well as trends in tissue oxygen saturation values measured at the right lower costal region and infraumbilical region differ, which could be explained in several ways. First, both sites have different origins of perfusion as stated before. Second, intestinal movements and passage of air and stools, influence mainly the infraumbilical measurements. Third, the inaccuracy of the NIRS technique and SomaSensors itself could have played a role. Fluctuations in oxygen saturation values have been reported, measured in the same infant and at the same location.[17] Because of the large discrepancies between liver and infraumbilical oxygen saturation measurements, it seems erratic to interchange $r_{liv}SO_2$ and $r_{int}SO_2$ values at a specific time point, thus limiting or even abolishing their potential for comparison and substitution in research or clinical practice. In the absence of a gold-standard for measuring splanchnic blood flow for a prolonged period of time, we were unable to assess the validity of the two NIRS measurements. We realize that this was a limitation of our study.

In conclusion, this study showed that the NIRS technique was feasible and safe for measuring tissue oxygen saturation in the liver and infraumbilical regions simultaneously in preterm infants with suspected NEC. Additionally, we demonstrated that the $r_{liv}SO_2$ and $r_{int}SO_2$ values were highly variable in time and that correlation and agreement between the

values is poor, which limits the potential for comparison and substitution. Further studies are needed to investigate the tissue or substance that is being measured by NIRS in the liver and infraumbilical regions and to explore the baseline values and trends of both liver and infraumbilical NIRS monitoring in preterm infants with abdominal symptoms and diseases. Furthermore, since the aim of this study was to investigate the feasibility of NIRS for monitoring in both the liver and infraumbilical regions simultaneously, and to compare the values obtained at the two locations, we did not assess the value of these measurements for predicting the onset of NEC. Larger groups of patients are required to determine the potential value of liver and/or infraumbilical NIRS monitoring in order to predict the onset and course of NEC in preterm infants.

ACKNOWLEDGMENTS

This study was part of the research program of the postgraduate school for Behavioural and Cognitive Neurosciences, University of Groningen. T.E.S. was financially supported by a grant from the Junior Scientific Master Class of the University of Groningen. We greatly acknowledge the help of Dr. Titia Brantsma-van Wulfften Palthe in Utrecht for correcting the English manuscript.

This study was funded by the Research Foundation of Beatrix Children's Hospital (BKZ10.1578), the Doelmatigheidsfonds (a cost-effectiveness fund) of University Medical Center Groningen (66940) and an unrestricted grant from the NutsOhra Foundation (1101-015).

REFERENCES

1. Wyatt JS, Cope M, Delpy DT, Wray S, Reynolds EO. Quantification of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet*. 1986;2(8515):1063-1066.
2. Fortune PM, Wagstaff M, Petros AJ. Cerebro-splanchnic oxygenation ratio (CSOR) using near infrared spectroscopy may be able to predict splanchnic ischaemia in neonates. *Intensive Care Med.* 2001;27(8):1401-1407.
3. Vanderhaegen J, Dehing L, Naulaers G, et al. Use of the liver tissue oxygenation index as a noninvasive parameter of intestinal ischemia in rabbits. *World J Surg.* 2007;31(12):2359-2362.
4. Stapleton GE, Eble BK, Dickerson HA, Andropoulos DB, Chang AC. Mesenteric oxygen desaturation in an infant with congenital heart disease and necrotizing enterocolitis. *Tex Heart Inst J.* 2007;34(4):442-444.
5. Gay AN, Lazar DA, Stoll B, et al. Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. *J Pediatr Surg.* 2011;46(6):1034-1040.
6. Kaufman J, Almodovar MC, Zuk J, Friesen RH. Correlation of abdominal site near-infrared spectroscopy with gastric tonometry in infants following surgery for congenital heart disease. *Pediatr Crit Care Med.* 2008;9(1):62-68.
7. Cortez J, Gupta M, Amaram A, Pizzino J, Sawhney M, Sood BG. Noninvasive evaluation of splanchnic tissue oxygenation using near-infrared spectroscopy in preterm neonates. *J Matern Fetal Neonatal Med.* 2011;24(4):574-582.
8. Petros AJ, Heys R, Tasker RC, Fortune PM, Roberts I, Kiely E. Near infrared spectroscopy can detect changes in splanchnic oxygen delivery in neonates during apneic episodes. *Eur J Pediatr.* 1999;158(2):173-174.
9. Teller J, Schwendener K, Wolf M, et al. Continuous monitoring of liver oxygenation with near infrared spectroscopy during nasogastric tube feeding in neonates. *Schweiz Med Wochenschr.* 2000;130(18):652-656.
10. Dave V, Brion LP, Campbell DE, Scheiner M, Raab C, Nafday SM. Splanchnic tissue oxygenation, but not brain tissue oxygenation, increases after feeds in stable preterm neonates tolerating full bolus orogastric feeding. *J Perinatol.* 2009;29(3):213-218.
11. Teller J, Wolf M, Keel M, Bucher HU, Fanconi S, Baenziger O. Can near infrared spectroscopy of the liver monitor tissue oxygenation? *Eur J Pediatr.* 2000;159(7):549.
12. Tokuka A, Tanaka A, Kitai T, et al. Interrelationship of oxygen supply by hepatic artery and portal vein: rapid analysis of ischemia reflow-induced changes in hepatic oxygenation in experimental and clinical subjects by tissue near-infrared spectroscopy. *Eur Surg Res.* 1994;26(6):342-352.
13. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg.* 1978;187(1):1-7.
14. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am.* 1986;33(1):179-201.
15. Pellicer A, Bravo Mdel C. Near-infrared spectroscopy: a methodology-focused review. *Semin Fetal Neonatal Med.* 2011;16(1):42-49.
16. McNeill S, Gatenby JC, McElroy S, Engelhardt B. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. *J Perinatol.* 2011;31(1):51-57.
17. Sorensen LC, Greisen G. Precision of measurement of cerebral tissue oxygenation index using near-infrared spectroscopy in preterm neonates. *J Biomed Opt.* 2006;11(5):054005.

CHAPTER 3

THE ASSOCIATION OF TISSUE OXYGENATION AND INTESTINAL FATTY ACID-BINDING PROTEIN IN PLASMA DURING THE DEVELOPMENT OF NECROTIZING ENTEROCOLITIS

Trijntje E. Schat*, Fardou H. Heida*, Maarten Schurink, Michelle E. van der Laan, Christian V. Hulzebos, Arend F. Bos, Elisabeth M.W. Kooi, Jan B.F. Hulscher

* Both authors contributed equally

Submitted

ABSTRACT

Background: The underlying pathophysiology of necrotizing enterocolitis (NEC) remains incompletely understood, particularly the role of intestinal perfusion.

Objectives: The main aim of the study was to determine the relation between cerebral and splanchnic fractional tissue oxygen extraction (FTOE) with intestinal fatty acid-binding protein in plasma (I-FABPp), a marker for intestinal damage, in infants with NEC. Furthermore, we investigated the courses of cerebral and splanchnic FTOE values and I-FABPp levels in uncomplicated (conservative treatment) and complicated NEC (surgery, or death).

Methods: We included 19 preterm infants with NEC (9 uncomplicated, 10 complicated). Using near-infrared spectroscopy, we measured regional cerebral and splanchnic tissue oxygen saturations continuously for 48 hours after NEC onset. We measured I-FABPp levels simultaneously. We used Spearman correlation tests to calculate correlation coefficients between FTOE values and I-FABPp levels in uncomplicated and complicated NEC.

Results: Median (range) gestational age was 28 (25-36) weeks and median (range) birth weight was 1290 (740-2400) grams. Cerebral and splanchnic FTOE values correlated strongly with I-FABPp levels (rho between .745 and .900; $P < .001$ - .037) during the first 16 hours after NEC onset. Thereafter, in uncomplicated NEC, splanchnic FTOE values increased while I-FABPp levels decreased concomitantly. In complicated NEC both splanchnic FTOE values and I-FABPp levels decreased.

Conclusion: Combining cerebral and splanchnic FTOE values with I-FABPp levels may enable us to discriminate between the progression of and recovery from intestinal damage in NEC.

INTRODUCTION

In preterm infants, necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal diseases.[1] The underlying pathophysiology of NEC remains incompletely understood, particularly the role of intestinal perfusion.[2,3]

The suggested role of impaired intestinal perfusion in NEC can be investigated by using near-infrared spectroscopy (NIRS). NIRS is a non-invasive method used increasingly in preterm infants to assess cerebral and intestinal perfusion.[4-9] NIRS measures regional tissue oxygen saturation (rSO_2) of underlying tissue continuously.[10] When transcutaneous arterial oxygen saturation (SpO_2) is measured simultaneously, fractional tissue oxygen extraction (FTOE) can be calculated.[11] FTOE is thought to reflect the balance between tissue oxygen supply and consumption and may, therefore, be an early indicator of impaired tissue perfusion.[11]

A possible way of gaining more insight into the role of cerebral and intestinal perfusion during the development of NEC is to combine FTOE values with intestinal fatty acid-binding protein (I-FABP), a marker for intestinal damage.[12,13] I-FABP is an intracellular protein located specifically in the epithelium of the small bowel. Following enterocyte damage, it is rapidly released into the circulation due, for example, to hypoxia.[12,13] In preterm infants with NEC, I-FABP levels in plasma (I-FABPp) are elevated and can serve as a predictor of the severity and extent of intestinal damage.[14-19]

Our first aim was to investigate whether FTOE values reflect intestinal damage due to NEC. We did so by relating cerebral and splanchnic FTOE values with I-FABPp levels in preterm infants with NEC. Secondly, we investigated whether, during the first 48 hours after NEC onset, the courses of cerebral and splanchnic FTOE values in relation to the course of I-FABPp levels differed in infants with uncomplicated and complicated NEC.

METHODS

Ethical approval

This study was part of a larger, prospective, observational cohort study registered with the Dutch Trial Registry under number NTR3239. The study was approved by the ethical review board of University Medical Center Groningen. Written informed parental consent was obtained in all cases.

Patient population

We included preterm infants admitted to the neonatal intensive care unit of University Medical Center Groningen between October 2010 and October 2012, who were suspected of or had recently been diagnosed with NEC. Suspected NEC was defined as the presence of non-specific NEC symptoms, such as bloody stools or distended abdomen. NEC was definitely confirmed if pneumatosis intestinalis, portal venous gas, or both were present according to the modified Bell's staging criteria.[20] As soon as a suspicion of NEC arose, the neonatologist put the patients on a nil per os and gastric decompression regime, and treated

them with broad-spectrum antibiotics until radiographic signs of NEC resolved and clinical signs stabilized.

The study design included measuring I-FABPp levels repeatedly, and measuring rSO_2 of cerebral and splanchnic tissue continuously, for 48 hours after NEC onset or until surgery, whichever came first. We defined NEC onset as the time of the first radiographic abdominal examination after clinical suspicion of NEC had arisen, including abdominal X-rays that had been taken in referring hospitals. After completing the study, a team of four consultants, blinded as to the results of the NIRS and I-FABP measurements, determined the modified Bell's staging criteria at NEC onset.[20] We also determined the end-stage Bell's stage. Agreement was reached in all cases.

In this paper we present data of the subgroup of preterm infants with definite NEC only, i.e., Bell's stage ≥ 2. We excluded those infants in whom NEC was not confirmed. We assigned the infants with NEC to one of two groups: those with uncomplicated NEC (conservative treatment sufficed) and those with complicated NEC (surgery was required, or the infant died). Indications for surgery, i.e. laparotomy, were bowel perforation (Bell's stage 3B) or lack of improvement despite optimal conservative therapy.

I-FABPp

With every routine blood analysis after definite NEC onset, an extra sample of 100 µL was obtained in an EDTA tube for study purposes. Ideally, blood samples were collected every 8 hours during the first 24 hours after definite NEC onset, and every 12 hours during the following 24 hours.

Blood samples were fractioned by centrifuging for 10 minutes at approximately 2000 x *g*. Plasma was then collected in a 5 mL Sarstedt tube and stored at -80°C. Next, a laboratory technician, who was blinded as to the clinical data, performed the I-FABPp measurements. We used the commercially available ELISAs for determining I-FABPp levels (Human FABP2 kit from R&D systems, Minneapolis, USA).

NIRS

We used the INVOS 5100C near-infrared spectrometer (Covidien, Mansfield, USA) in combination with the neonatal SomaSensors (Covidien) to measure cerebral and splanchnic oxygen saturation continuously for 48 hours after NEC onset.

To measure cerebral tissue oxygen saturation (r_cSO_2), we placed the neonatal SomaSensor on the right or left frontoparietal side of the infant's head. We measured splanchnic oxygen saturation at two locations: just below the right costal arch to measure liver oxygen saturation ($r_{liv}SO_2$), and infraumbilically on the central abdomen to measure intestinal oxygen saturation ($r_{int}SO_2$). The SomaSensors were held in place by elastic bandaging or Mepitel (Mölnlycke, Sweden). During routine nursing care, clinical assessments, and radiographic examinations the sensor was temporarily removed. Afterwards, it was replaced in the same location. The data, collected prospectively, were stored off-line for future analysis.

Additionally, we collected SpO_2 (Nellcor, Covidien), and calculated FTOE using the formula: FTOE = (SpO_2 - rSO_2) / SpO_2.[11] FTOE reflects the balance between tissue oxygen delivery and tissue oxygen consumption.[11] Changes in FTOE reflect changes in tissue perfusion. In this way tissue hypoxia can be detected.

Demographic and clinical variables

We collected the following patient characteristics from patient reports: gestational age, birth weight, postnatal age at NEC onset, gender, whether surgery was required, and mortality. Furthermore, we documented the first concentrations during the study period of hemoglobin, thrombocytes, pH, C-reactive protein, and lactate. Additionally, we documented the need for mechanical ventilation, treatment of circulatory failure (volume expansion, vasoactive drugs), and the presence of a (hemodynamically significant) patent ductus arteriosus during the study period. It was defined as a diastolic forward flow in the branches of the pulmonary artery, a diastolic backflow in the descending aorta, and a left ventricular end diastolic diameter > p 95.

Data and statistical analysis

For the first aim of this study, we correlated cerebral and splanchnic FTOE values with I-FABPp levels in preterm infants with Bell's stage ≥ 2. Subsequently, we analyzed the courses of cerebral and splanchnic FTOE values in association with I-FABPp levels in preterm infants in whom NEC developed without complications and in preterm infants with complicated NEC. Cerebral and splanchnic regional tissue oxygen saturations were collected once every 6 seconds, whilst SpO_2 values were collected once every 5 minutes. We matched each SpO_2 value with the corresponding single rSO_2 value, leaving one coupled measurement every 5 minutes. Next, we calculated FTOE values using these combined SpO_2 and rSO_2 values for the cerebral, liver, and intestinal regions separately.

Since I-FABPp levels were collected once every 8 hours in the first 24 hours after NEC onset and once every 12 hours between 24 and 48 hours after NEC onset, we calculated 8-hour mean FTOE values in the first 24 hours after NEC onset and subsequently 12-hour mean FTOE values for the remaining study period. Next, we calculated correlation coefficients between cerebral and splanchnic FTOE values and I-FABPp levels during the first 48 hours after NEC onset using the Spearman rank correlation test.

For our second aim, we constructed courses of cerebral and splanchnic FTOE values together with I-FABPp levels for infants with uncomplicated NEC and complicated NEC after logarithmic transformation of the I-FABPp levels.

Categorical data were tested by the chi-square test or Fischer's exact test and continuous data by the Mann-Whitney test. We used SPSS 22.0 software for Windows (IBM SPSS Statistics 22, IBM Corp., Armonk, New York, USA) for the statistical analyses. We considered a *P* value < .05 statistically significant.

RESULTS

Patient characteristics

We enrolled 19 preterm infants with NEC Bell's stage ≥ 2 in whom we were able to measure cerebral and splanchnic tissue oxygen saturation simultaneously and collect plasma for analyzing I-FABP levels (Figure 1). I-FABPp levels could not be assessed in cases in which the condition of the infant was too critical to obtain routine blood analysis. The median (range) time between NEC onset and the beginning of NIRS monitoring was 7 (2-31) hours. NIRS data could not be assessed in cases of incorrect sensor placement, or for logistic reasons when NIRS devices were not available.

In nine infants the course of NEC was uncomplicated. One of these infants required surgery as a result of post-NEC stricture 74 days after NEC onset. Of the ten infants with complicated NEC, two infants who were diagnosed with Bell's stage 3A, died as a consequence 5 and 35 days after NEC onset. Eight infants were found to have Bell's stage 3B, with bowel perforation. Eight infants with complicated NEC required surgery; seven of them were operated on during the study period, with a median of 33 hours (range, 9-165) between the onset of NEC symptoms and surgery. We present the patient characteristics in Table 1.

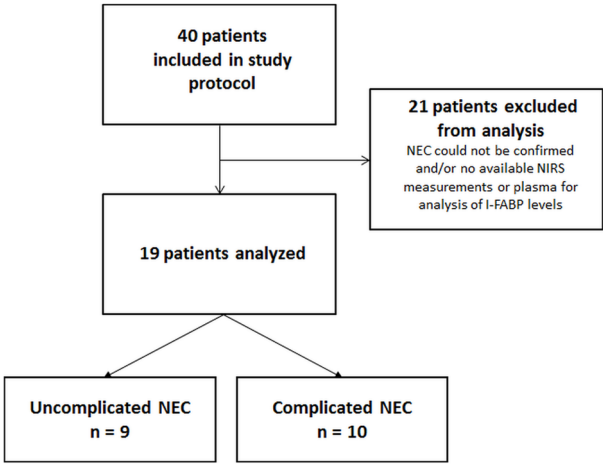

Figure 1. Flow diagram of the study.

The correlation between cerebral and splanchnic FTOE values and I-FABPp levels

In Table 2 we present the correlation coefficients between cerebral and splanchnic FTOE values and I-FABPp levels. We found strong correlation coefficients between these variables during the first 16 hours after NEC onset (Table 2). Additionally, cerebral FTOE values correlated significantly with I-FABPp levels between 24 and 36 hours after NEC onset (Table 2).

Table 1. Patient characteristics.

	Uncomplicated NEC (n = 9)	Complicated NEC (n = 10)	*P* value
Gestational age, weeks	31.6 (25.7-35.9)	26.7 (25.0-34.0)	.025*
Birth weight, grams	1520 (740-2400)	980 (790-2280)	.050
Male:Female	6:3	8:2	.628
PNA at NEC diagnosis, days	8 (3-29)	9 (7-22)	.589
Hemoglobin, mmol/L	8.7 (7.0-12.4)	8.2 (6.0-10.3)	.567
Thrombocytes, 10^9/L	235 (131-491)	202 (42-405)	.142
pH	7.34 (7.19-7.39)	7.24 (7.09-7.42)	.130
C-reactive protein, mg/L	33 (0-166)	30 (0-95)	.838
Lactate, mmol/L	2.7 (1.2-4.5) (n = 4)	2.0 (1.0-11.9) (n = 8)	.799
Mechanical ventilation (%)	3 (33)	7 (70)	.179
PDA (%)	1 (11)	3 (30)	.582
Hemodynamically significant (%)	-	2 (20)	.474
RBC transfusion (%)	3 (33)	4 (40)	.999
Fluid resuscitation (%)	4 (44)	8 (80)	.170
Inotropes (%)	-	6 (60)	.011*
Surgery (%)	1 (11)	8 (80)	.005*
Mortality (%)	-	6 (60)	.011*

Data are expressed as median (range) or as numbers unless specified otherwise.

Abbreviations: NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; PNA, postnatal age; RBC, red blood cell.

Statistical differences between the two groups are marked by * (< .05)

The courses of cerebral and splanchnic FTOE values and I-FABPp levels in infants with complicated and uncomplicated NEC

Figure 2 shows the courses of cerebral, liver, and intestinal FTOE values and I-FABPp levels in preterm infants with uncomplicated NEC and infants with complicated NEC. During the first 16 hours after NEC onset we found little difference in the courses of cerebral and splanchnic FTOE values and I-FABPp levels between infants with uncomplicated and complicated NEC. From 16 hours after NEC onset, however, we found that both splanchnic FTOE values and I-FABPp levels decreased in preterm infants with complicated NEC. In preterm infants with uncomplicated NEC we observed the opposite: splanchnic FTOE values gradually increased whilst I-FABPp levels decreased. We observed this increase and decrease of splanchnic FTOE values particularly in the intestinal region, as compared to the liver region.

Please note that we constructed these courses using all the data available for the included infants, even though the FTOE values and/or I-FABPp levels were not available at each time period for every infant.

Table 2. Correlation coefficients between cerebral and splanchnic FTOE values and I-FABP levels in plasma.

	cFTOE	livFTOE	intFTOE
0-8 hours			
I-FABPp	.786 n = 7 *P* = .036*	.600 n = 5 *P* = .285	.900 n = 5 *P* = .037*
8-16 hours			
I-FABPp	.891 n = 11 *P* < .001**	.881 n = 8 *P* = .004*	.745 n = 10 *P* =.013*
16-24 hours			
I-FABPp	.750 n = 7 *P* = .052	.771 n = 6 *P* = .072	.800 n = 4 *P* = .200
24-36 hours			
I-FABPp	.731 n = 13 *P* = .005*	.573 n = 11 *P* = .066	-.285 n = 10 *P* = .425
36-48 hours			
I-FABPp	.510 n = 12 *P* = .090	.100 n = 11 *P* = .770	.188 n = 10 *P* = .603

cFTOE, cerebral fractional tissue oxygen extraction; I-FABPp, intestinal fatty acid-binding protein in plasma; intFTOE, intestinal fractional tissue oxygen extraction; livFTOE, liver fractional tissue oxygen extraction.
Statistical differences are marked by * (< .05) or ** (< .001).

DISCUSSION

Our results demonstrated a strong association between cerebral and splanchnic FTOE values on the one hand and I-FABPp levels on the other hand during the first 16 hours after NEC onset. Furthermore, we observed distinct patterns of FTOE values and I-FABPp levels during the first 48 hours in the development of NEC between infants with uncomplicated and complicated NEC. From 16 hours after NEC onset, we observed decreasing splanchnic FTOE values and I-FABPp levels in infants with complicated NEC, whilst infants with uncomplicated NEC showed increasing splanchnic FTOE values concomitant with decreasing I-FABPp levels. Pathologically, NEC is characterized by coagulation necrosis of the intestinal wall, which suggests that intestinal ischemia plays a role in its pathogenesis.[21] Given that NIRS is a non-invasive bedside tool providing information about tissue perfusion, it may be useful for assessing the perfusion status of preterm infants with NEC.[22] Our data suggested that high splanchnic FTOE values did indeed reflect low intestinal perfusion with hypoxia during the early development of NEC, because they concurred with high levels of I-FABPp, a marker for intestinal damage.[12,13]

We offer several explanations for the strong positive associations between splanchnic FTOE values and I-FABPp levels during the first 16 hours after NEC onset. First, the presence of

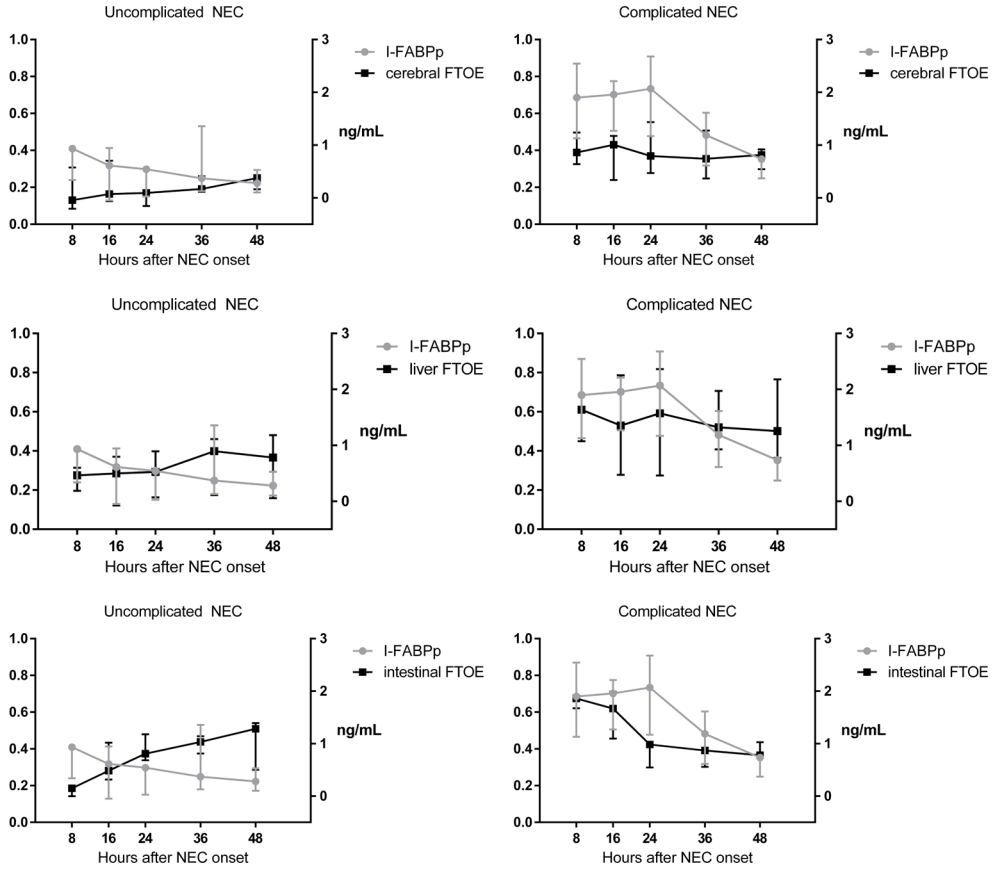

Figure 2. Graphs showing the median (dots and squares) and interquartile range (lines) of cerebral and splanchnic FTOE values and I-FABPp levels of infants with uncomplicated and complicated NEC.

ischemia may cause intestinal epithelial cell damage. Second, intestinal ischemia and hypoxia may develop as a consequence of circulatory insufficiency in the presence of intestinal epithelial cell damage. Finally, intestinal circulation may be affected locally as a result of intestinal injury. Whatever the case may be, our data suggested that splanchnic FTOE values can be used to gain information about the degree of intestinal damage during NEC.

Interestingly, we not only found strong correlations between splanchnic FTOE values and I-FABPp levels, but also between cerebral FTOE values and I-FABPp levels. This finding may be a reflection of the fact that by the time NEC becomes clinically evident, it has already had systemic effects on hemodynamics. In this case, systemic circulatory insufficiency, e.g., the need for volume expansion and/or inotropes, may have occurred earlier on, compromising cerebral perfusion as well.[23] Another explanation is that cerebrovascular autoregulation in these preterm infants might have been compromised.[24,25]

In all infants with NEC, irrespective of whether the disease developed with or without complications, we found decreasing I-FABPp levels 16 hours after NEC onset. This may be the result of one of two mechanisms: either expansion of damage or recovery of intestinal tissue. In case of complicated NEC, it could be caused by intestinal necrosis leaving no villi to secrete I-FABP or the absence of blood flow through a demarcated necrotic bowel segment .[13,19] Conversely, when the infant's condition is ameliorating and the intestinal tissue is not injured any further, secretion of I-FABP into the circulation will diminish.[19] On the basis of concentrations of I-FABPp levels alone we cannot differentiate between these two hypothesized mechanisms. With simultaneous knowledge of FTOE values, however, we were able to differentiate between the aforementioned supposed mechanisms. We identified two distinct patterns during the 48 hours during which the disease was developing, one predominant in infants with uncomplicated NEC and the other in infants with complicated NEC.

In infants with uncomplicated NEC, we observed low splanchnic FTOE values during the first 16 hours after NEC onset that increased during the remainder of the study period. We hypothesize that hyperemia is present during the first hours after NEC onset due to an inflammatory response. As time progresses, hyperemia gradually disappears, which explains the increasing FTOE values. We hypothesize that this course of FTOE values in combination with decreasing I-FABPp levels represents recovery of intestinal tissue.

We observed the opposite in infants with complicated NEC, i.e., relatively high initial splanchnic FTOE values, and gradually decreasing intestinal FTOE as the disease developed. We speculate that the high splanchnic FTOE values during the first 16 hours after NEC onset were the result of compromised intestinal perfusion. From 16 hours after NEC onset, however, the splanchnic FTOE values decreased, which suggests decreasing or absent intestinal metabolism due to the presence of necrotic bowel. In these infants, therefore, decreasing I-FABPp levels could possibly have been the result of increasing intestinal injury. In the intestinal region the increase and decrease of FTOE values was more distinct than in the region of the liver. This could be explained by the liver's unique blood supply - in addition of receiving partially deoxygenated blood from the intestinal region through the portal vein, it also receives oxygenated blood from the hepatic artery.[26]

The strength of this study was that we obtained plasma samples at regular intervals and that we measured the cerebral and splanchnic rSO_2 values with NIRS simultaneously. Furthermore, we measured cerebral and splanchnic oxygenation for 48 consecutive hours. Finally, this was the first study to correlate FTOE values with a marker for intestinal damage. A limitation of this study was the relatively small sample size. Nevertheless, we found very strong associations between cerebral and splanchnic FTOE and I-FABPp levels. Moreover, we included all available data, including data of infants for whom FTOE values and/or I-FABPp levels were not available for each time period. This might have led to a selection bias. Finally, we did not include a control group. However, the aim of this study was to investigate whether intestinal perfusion plays a role in the course of NEC, and, together with I-FABPp

measurements differentiated between a complicated or uncomplicated course. Future research should also investigate differences in intestinal perfusion measures between infants with NEC and relatively healthy and stable preterm infants.

Our findings may have clinical implications. They suggested that impaired intestinal perfusion played a pivotal role in the development of complications during the early stages of the disease. Combined measurements of splanchnic FTOE values and I-FABPp levels in the initial phase of NEC might indicate individual infants who are at high risk of developing intestinal perforations. Early detection of impaired intestinal perfusion and hypoxia would be most helpful, because the assessment of intestinal necrosis and the timing of surgery for NEC, especially in the absence of perforation, remain difficult. It may also lead to new interventions, other than surgical ones, aimed at counteracting the progression of NEC into complicated disease. Further research is warranted to confirm this hypothesis.

CONCLUSION

We found strong associations between FTOE values of cerebral and splanchnic tissue on one hand and I-FABPp levels on the other during the first 16 hours after NEC onset, suggesting that FTOE values can be used to gain information about the degree of intestinal damage. Additionally, during the first 48 hours after NEC onset, we identified distinct splanchnic FTOE and I-FABPp courses in preterm infants with uncomplicated NEC and complicated NEC. This finding suggests that impaired intestinal perfusion and hypoxia play an important role early on in the development of complicated NEC.

ACKNOWLEDGMENTS

This study was part of the research program of the Graduate School of Medical Sciences, the Groningen University Institute for Drug Exploration, and the postgraduate school for Behavioural and Cognitive Neurosciences, University of Groningen. T.E. Schat, F.H. Heida, and M.E. van der Laan were financially supported by a grant from the Junior Scientific Master Class of University of Groningen. Finally, we greatly acknowledge the help of Dr. T. van Wulfften Palthe for correcting the English manuscript.

REFERENCES

1. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011;364(3):255-264.
2. Nowicki PT. Ischemia and necrotizing enterocolitis: Where, when, and how. *Semin Pediatr Surg*. 2005;14(3):152-158.
3. Watkins DJ, Besner GE. The role of the intestinal microcirculation in necrotizing enterocolitis. *Semin Pediatr Surg*. 2013;22(2):83-87.
4. Cortez J, Gupta M, Amaram A, Pizzino J, Sawhney M, Sood BG. Noninvasive evaluation of splanchnic tissue oxygenation using near-infrared spectroscopy in preterm neonates. *J Matern Fetal Neonatal Med*. 2011;24(4):574-582.
5. Gay AN, Lazar DA, Stoll B, et al. Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. *J Pediatr Surg*. 2011;46(6):1034-1040.
6. McNeill S, Gatenby JC, McElroy S, Engelhardt B. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. *J Perinatol*. 2011;31(1):51-57.
7. Gillam-Krakauer M, Cochran CM, Slaughter JC, et al. Correlation of abdominal rSO2 with superior mesenteric artery velocities in preterm infants. *J Perinatol*. 2013;33(8):609-612.
8. Pellicer A, Greisen G, Benders M, et al. The SafeBoosC phase II randomised clinical trial: A treatment guideline for targeted near-infrared-derived cerebral tissue oxygenation versus standard treatment in extremely preterm infants. *Neonatology*. 2013;104(3):171-178.
9. Patel AK, Lazar DA, Burrin DG, et al. Abdominal near-infrared spectroscopy measurements are lower in preterm infants at risk for necrotizing enterocolitis. *Pediatr Crit Care Med*. 2014;15(8):735-741.
10. Wyatt JS, Cope M, Delpy DT, Wray S, Reynolds EO. Quantification of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet*. 1986;2(8515):1063-1066.
11. Naulaers G, Meyns B, Miserez M, et al. Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation. A validation study in piglets. *Neonatology*. 2007;92(2):120-126.
12. Gollin G, Marks C, Marks WH. Intestinal fatty acid binding protein in serum and urine reflects early ischemic injury to the small bowel. *Surgery*. 1993;113(5):545-551.
13. Lieberman JM, Sacchettini J, Marks C, Marks WH. Human intestinal fatty acid binding protein: Report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery*. 1997;121(3):335-342.
14. Edelson MB, Sonnino RE, Bagwell CE, Lieberman JM, Marks WH, Rozycki HJ. Plasma intestinal fatty acid binding protein in neonates with necrotizing enterocolitis: A pilot study. *J Pediatr Surg*. 1999;34(10):1453-1457.
15. Evennett N, Alexander N, Petrov M, Pierro A, Eaton S. A systematic review of serologic tests in the diagnosis of necrotizing enterocolitis. *J Pediatr Surg*. 2009;44(11):2192-2201.
16. Aydemir C, Dilli D, Oguz SS, et al. Serum intestinal fatty acid binding protein level for early diagnosis and prediction of severity of necrotizing enterocolitis. *Early Hum Dev*. 2011;87(10):659-661.
17. Schurink M, Scholten IG, Kooi EM, et al. Intestinal fatty acid-binding protein in neonates with imminent necrotizing enterocolitis. *Neonatology*. 2014;106(1):49-54.
18. Heida FH, Hulscher JB, Schurink M, et al. Intestinal fatty acid-binding protein levels in necrotizing enterocolitis correlate with extent of necrotic bowel: Results from a multicenter study. *J Pediatr Surg*. 2014.

19. Schurink M, Kooi EM, Hulzebos CV, et al. Intestinal fatty acid-binding protein as a diagnostic marker for complicated and uncomplicated necrotizing enterocolitis: A prospective cohort study. *PLoS One*. 2015;10(3):e0121336.
20. Walsh MC, Kliegman RM. Necrotizing enterocolitis: Treatment based on staging criteria. *Pediatr Clin North Am*. 1986;33(1):179-201.
21. Ballance WA, Dahms BB, Shenker N, Kliegman RM. Pathology of neonatal necrotizing enterocolitis: A ten-year experience. *J Pediatr*. 1990;117(1 Pt 2):S6-13.
22. Moore JE. Newer monitoring techniques to determine the risk of necrotizing enterocolitis. *Clin Perinatol*. 2013;40(1):125-134.
23. Hanson SJ, Berens RJ, Havens PL, Kim MK, Hoffman GM. Effect of volume resuscitation on regional perfusion in dehydrated pediatric patients as measured by two-site near-infrared spectroscopy. *Pediatr Emerg Care*. 2009;25(3):150-153.
24. Greisen G. Autoregulation of cerebral blood flow in newborn babies. *Early Hum Dev*. 2005;81(5):423-428.
25. Verhagen EA, Hummel LA, Bos AF, Kooi EM. Near-infrared spectroscopy to detect absence of cerebrovascular autoregulation in preterm infants. *Clin Neurophysiol*. 2014;125(1):47-52.
26. Teller J, Wolf M, Keel M, Bucher HU, Fanconi S, Baenziger O. Can near infrared spectroscopy of the liver monitor tissue oxygenation? *Eur J Pediatr*. 2000;159(7):549.

CHAPTER 4

ASSESSING CEREBROVASCULAR AUTOREGULATION IN INFANTS WITH NECROTIZING ENTEROCOLITIS USING NEAR-INFRARED SPECTROSCOPY

Trijntje E. Schat, Michelle E. van der Laan, Maarten Schurink,
Jan B.F. Hulscher, Christian V. Hulzebos,
Arend F. Bos, Elisabeth M.W. Kooi

Pediatric Research, provisionally accepted for publication

ABSTRACT

Background: We assessed cerebrovascular autoregulation (CAR) in preterm infants with definite necrotizing enterocolitis (NEC), Bell's stage 2 or 3, and infants without NEC, using near-infrared spectroscopy (NIRS). We hypothesized that CAR would be more often impaired in infants with NEC compared with infants without NEC.

Methods: We measured cerebral regional tissue oxygen saturation, arterial oxygen saturation, and mean arterial blood pressure (MABP) during 48 hours. We calculated the correlation between cerebral fractional tissue oxygen extraction and MABP for each patient. A statistically significant negative correlation reflected impaired CAR.

Results: We included fifteen infants with definite NEC (median (range) gestational age (GA) 27.4 (25.6-34.7) weeks, birth weight (BW) 1070 (670-2400) grams) and thirteen infants without NEC (GA 27.9 (26.3-34.7) weeks, BW 980 (640-2640) grams). Fourteen infants had a statistically significant negative correlation (rho -.468 to -.104), of whom five were infants without NEC (5/13; 38%) and nine with definite NEC (9/15; 60%). The difference in prevalence of impaired CAR was not statistically significant.

Conclusion: Impaired CAR is present in a substantial proportion of infants with definite NEC, which may predispose them to NEC-associated neurological damage.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease that predominantly affects preterm infants.[1] Several studies demonstrated a strong association between NEC and impaired neurodevelopmental outcome.[2-7] Moreover, neurodevelopmental delay in preterm infants with NEC assessed at school age was found to be associated with more white matter abnormalities as seen on magnetic resonance imaging at term compared to infants who were also born prematurely but who did not have NEC.[8] White matter abnormalities are considered to be the principal anatomic substrate for the neurodevelopmental disability in infants with NEC.[9]

The development of white matter injury depends on the occurrence of two separate mechanisms: infection and/or inflammation and ischemia.[10] Infants with NEC develop an excessive inflammatory response with production of inflammatory toxins.[11] This may affect systemic circulation, and – if severe – also cerebral circulation. Ischemia of cerebral tissue is the result of diminished cerebral blood flow (CBF) caused, amongst others, by impaired cerebrovascular autoregulation (CAR). CAR plays a pivotal role in regulating CBF. When CAR is affected, a pressure-passive cerebral circulation arises, i.e. changes in blood pressure cause changes in CBF. When CBF is low, underperfusion of cerebral tissue ensues, which may lead to cerebral ischemia and subsequent brain damage.

Our research group demonstrated that near-infrared spectroscopy (NIRS) can be used to estimate the presence or absence of CAR in otherwise stable preterm infants, by assessing the relationship between mean arterial blood pressure (MABP) and cerebral fractional tissue oxygen extraction (FTOE). We assumed that a statistically significant negative correlation between MABP and cerebral FTOE reflects impaired CAR. CAR was found to be impaired in 40% of relatively stable preterm infants, also in infants where blood pressures were in the 'normal' range.[12] In the absence of CAR, the relationship between MABP and cerebral perfusion is linear. Because FTOE is a reflection of cerebral perfusion, changes in MABP cause opposite changes in cerebral FTOE if CAR is impaired. This linear relationship is independent of the values measured and, as such, can also occur in the presence of blood pressures in the 'normal' range.

It is unknown what the prevalence is of impaired CAR in preterm infants with NEC and whether CAR is impaired more often in preterm infants with established NEC than in infants without NEC. Our aim was, therefore, to assess cerebral hemodynamics using NIRS in preterm infants with NEC (Bell's stage 2 or 3) and without NEC, and to compare the prevalence of impaired CAR between these two groups. We hypothesized that definite NEC would be associated with a higher prevalence of impaired CAR in comparison to infants without NEC, due to the presence of infection/inflammation leading to an affected cerebral circulation.

METHODS

Study population

We included infants with definite NEC who were part of a larger, prospective observational cohort study performed in the neonatal intensive care unit of University Medical Center Groningen, between October 2010 and October 2012. This study was registered in the Dutch Trial Registry under number NTR3239. Infants who were suspected of NEC or who had been diagnosed with NEC were included. Suspected NEC was defined as the presence of non-specific abdominal symptoms. NEC was diagnosed when pneumatosis intestinalis, portal venous gas, or both were present on abdominal radiographic examination. After completion of the study, an expert panel of consultant neonatologists and pediatric surgeons independently classified the infants into the modified Bell's stages.[13] Consensus was reached in all cases. For the purpose of this study, we included only those preterm infants with a continuous invasive arterial blood pressure measurement who were diagnosed with definite NEC. We defined the onset of NEC symptoms as the time of the first abdominal radiographic examination taken after clinical suspicion of NEC, including those taken in referring hospitals. The study was approved by the institutional ethics review board of University Medical Center Groningen. Written parental informed consent was obtained in all cases.
We selected control infants that were admitted to our neonatal intensive care unit between October 2010 and December 2012. The attending neonatologist decided on NIRS monitoring on clinical grounds. We only included infants with a gestational age (GA) < 35 weeks, with an indwelling arterial catheter for constant blood pressure measurements. Exclusion criteria were the presence of NEC or sepsis.

Near-infrared spectroscopy

We measured cerebral regional tissue oxygen saturation (r_cSO_2) continuously with a near-infrared spectrometer (INVOS 5100C, Covidien, Mansfield, MA, USA). The neonatal Somasensor (Covidien) was placed on the frontoparietal side of the infant's head and was secured with elastic bandaging or Mepitel (Mölnlycke, Sweden).
The sensor emits two wavelengths (730 and 810 nm) in the cerebral tissue. The two receivers, at 30 and 40 mm distance from the emitter, receive the reflected light as a function of wavelength. Hence, the spectral absorption of the underlying tissue can be determined.[14,15] Since oxygenated and deoxygenated hemoglobin each absorb and reflect near-infrared light at the two wavelengths differently, it is possible to calculate regional tissue oxygen saturation, i.e. microvascular oxygenation.
We measured arterial oxygen saturation (SpO_2) (Nellcor, Covidien) simultaneously with r_cSO_2 and calculated cerebral FTOE using the formula: FTOE = SpO_2 - r_cSO_2 / SpO_2.[16] FTOE reflects the balance between tissue oxygen supply and tissue oxygen consumption. As cerebral oxygen consumption is thought to be relatively stable in preterm infants, FTOE can be used as an indicator of tissue perfusion.[16]

Analysis of cerebrovascular autoregulation

A blood pressure dependent CBF (pressure-passive CBF) can be the result of two mechanisms: blood pressures below the autoregulatory threshold or impaired CAR. Cerebral FTOE was found to be inversely related to CBF.[17] In the presence of impaired CAR, FTOE will also be blood pressure dependent. In that case, low blood pressures cause higher cerebral oxygen extraction in order to meet the needs for normal cerebral metabolism. We used this negative relationship between arterial blood pressure and cerebral FTOE to assess the absence of CAR. We considered a statistically significant negative correlation coefficient as indicating impaired CAR.[12]

Clinical data

We collected the following neonatal demographic and clinical characteristics: birth weight (BW), GA, postnatal age at first NIRS measurement, the concentration of hemoglobin (Hb), glucose, carbon dioxide (pCO_2), and C-reactive protein (CRP), the need of mechanical ventilation, and mortality. Additionally, we documented the administration of red blood cell transfusions, volume expansion, and inotropes, and the presence of a hemodynamically significant PDA during the study period. Hemodynamically significant patent ductus arteriosus (PDA) was defined as a diastolic forward flow in the branches of the pulmonary artery, a diastolic backflow in the descending aorta, and a left ventricular end diastolic diameter > the 95th centile.

We determined Hb and pCO_2 as the first value just before or during the NIRS recording period. The glucose concentration is based on the lowest value during NIRS recording, while the CRP concentration is based on the highest value during NIRS recording.

Statistics

In infants with NEC, r_cSO_2 recording started as soon as possible after clinical signs and symptoms suggested the presence of NEC. We sampled one random value of r_cSO_2, SpO_2, FTOE, and MABP every 5 minutes for 48 consecutive hours for both infants with NEC and control infants. Next, we calculated 48-hour mean values for each variable. Artifacts in the measurements were not taken into the analyses. The Spearman rank test was used to calculate the correlation coefficients between MABP and cerebral FTOE, sampled every 5 minutes, for each preterm infant individually. Next, we compared the prevalence of impaired CAR between infants without NEC and infants with definite NEC (Bell's stage 2 or 3) using Fisher exact test.

We compared proportions of categorical data with Fisher exact test and median values were analyzed by using the Mann-Whitney test for non-normal distributions. We used SPSS 22.0 software for Windows (IBM SPSS Statistics 22, IBM Corp., Armonk, New York, USA) for all our statistical analyses. A *P* value of < .05 was considered statistically significant.

RESULTS

We included fifteen infants with definite NEC and thirteen control patients. We present the patient characteristics for the two groups in Table 1. Infants with NEC had significantly higher pCO_2 and CRP concentrations. NIRS monitoring in infants with NEC had started significantly later compared with the control group. Furthermore, infants with definite NEC received inotropes, volume expansion, and RBC transfusions significantly more often during the study period compared with controls.

Of the six infants with NEC who died, four infants did so as a result of circulatory and respiratory insufficiency within the 48-hour study period. The fifth infant died due to circulatory insufficiency ten days after the NIRS measurements - most likely as a result of an anastomotic leak. The sixth infant died 89 days after the study period due to irreversible brain damage, which most likely occurred during surgery for an intestinal obstruction. The infant without NEC who died, did so 66 days after the study period, due to bronchopulmonary dysplasia.

In the infants with NEC, NIRS recordings started after a median time of 11 hours (range, 2-29) after onset of NEC symptoms and were discontinued preliminary in six infants due to the presence of pneumoperitoneum on abdominal radiographic examination.

Out of the fifteen infants in the NEC group, we found nine (60%) with a statistically significant negative correlation between MABP and cerebral FTOE. Out of the thirteen infants without NEC, we found five infants (38%) with a statistically significant negative correlation. This difference was not statistically significant ($P = .449$). Table 2 shows the correlation coefficients with associated *P* values per infant.

In Figure 1 we present the correlation coefficients between cerebral FTOE and MABP for one infant with NEC with a statistically significant negative correlation suggesting impaired CAR and for one infant without NEC without a statistically significant negative correlation suggesting adequate CAR. The infant with definite NEC was monitored for 8 hours until pneumoperitoneum was detected on abdominal radiographic examination. The infant was taken to theatre shortly afterwards.

DISCUSSION

We demonstrated that 60% of preterm infants with definitive NEC (Bell's stage 2 and 3) had a statistically significant negative correlation between cerebral FTOE and MABP, suggesting impaired CAR. This is a high percentage in comparison to the preterm infants without NEC (38%). Nevertheless, this difference was not statistically significant.

For preterm infants who suffered NEC, the short-term and long-term neurological sequelae can be devastating. Timely identification of infants with impaired CAR is, therefore, important in order to prevent the development of white matter abnormalities. Our data indicate that, to this end, NIRS is a useful, non-invasive, bedside monitoring method.

Several studies investigated the presence of CAR in preterm infants using near-infrared spectroscopy.[12,18-22] The prevalence of pressure-passivity ranged from 14 to 53%. These

Table 1. Patient characteristics of infants without NEC and infants with definite NEC.

	No NEC (n = 13)	Definite NEC (n = 15)
Gestational age (weeks)	27.9 (26.3-34.7)	27.4 (25.6-34.7)
Birth weight (grams)	980 (640-2640)	1070 (670-2400)
Postnatal age (days)	4 (3-13)	10 (3-34)*
Male/Female	6/7	10/5
Bell's stage 2/Bell's stage 3	N/A	5/10
Hemoglobin (mmol/L)	8.3 (7.3-10.5) (n = 10)	7.4 (6.0-12.0)
pCO_2 (kPa)	5.6 (4.8-6.6) (n = 10)	6.5 (4.4-10.1)*
Glucose (mmol/L)	4 (3.2-7) (n = 7)	6.5 (3.2-8.6)
CRP (mg/L)	0 (0-8) (n = 7)	110 (7-425)**
Mechanical ventilation (%)	6 (46)	12 (80)
hsPDA (%)	4 (31)	1 (7)
Inotropes (%)	0 (-)	7 (47)*
Volume expansion (%)	1 (8)	12 (80)**
RBC transfusion (%)	1 (8)	8 (53)*
Mortality (%)	1 (8)	6 (40)

Data are expressed as median (range) or as numbers unless specified otherwise.

Abbreviations: hsPDA - hemodynamically significant patent ductus arteriosus; N/A - not applicable; RBC - red blood cell.

Statistical differences between the two groups are marked by * (< .05) or ** (< .001).

studies, however, all used different parameters to assess CAR and also performed their analyses differently, a fact which complicates any comparison. Our definition of impaired CAR was based on a study performed previously in our research group. Verhagen *et al.* demonstrated that 40% of clinically stable preterm infants had a statistically significant negative correlation between cerebral FTOE and MABP.[12] We found similar results in our control group.

In preterm infants with definite NEC, we found a statistically significant negative correlation, which suggested impaired CAR, between cerebral FTOE and MABP in 60% of cases. A possible explanation for this higher percentage could be that preterm infants with NEC might have had blood pressures under the lower limit of the autoregulatory curve, with a consequential pressure-passive CBF. We did not find statistically significant negative correlations between cerebral FTOE and MABP for those infants who had the lowest mean MABP. Indeed, it was suggested that impaired CAR might develop when blood pressures are in the range of what we now define as 'normal' and 'safe'.[12,19] In these instances, it is of the utmost importance to only allow minimal decreases in blood pressure in order to prevent low cerebral perfusion and the subsequent risk of developing brain injury.

Factors that were found to be associated with impaired CAR are low BW,[19,20] low GA,[20] low Hb,[23] low glucose,[24] and high pCO_2 concentrations.[25,26] PCO_2 concentrations were significantly higher in preterm infants with NEC, which suggests a possible contributing role of pCO_2 in causing pressure-passive CBF in the preterm infants with NEC we studied. Nevertheless, we would like to stress that in our study group the pCO_2 values were based on single

Table 2. Individual correlation coefficients between cerebral FTOE and MABP, sampled every five minutes.

Infant	Bell's stage	Measurement time in hours	MABP	FTOE	Spearman's rho	*P* value
1	NA†	23	33	.16	-.374	.000
2	NA	38	31	.34	-.271	.000
3	NA	32	42	.36	-.208	.000
4	NA	40	32	.24	-.173	.000
5	NA	45	50	.31	-.104	.016
6	NA	26	38	.27	-.077	NS
7	NA	32	30	.22	-.047	NS
8	NA	46	33	.34	-.034	NS
9	NA	15	48	.30	-.005	NS
10	NA	32	41	.09	.063	NS
11	NA	43	38	.22	.222	.000
12	NA	44	43	.15	.227	.000
13	NA	10	40	.15	.263	.003
14	3	8	34	.48	-.468	.000
15	3	34	30	.44	-.292	.000
16	2	43	39	.26	-.284	.000
17	3†	47	29	.34	-.275	.000
18	2	29	58	.05	-.270	.000
19	3†	27	32	.42	-.258	.000
20	3†	2	46	.43	-.237	NS
21	2	30	45	.15	-.150	.004
22	3†	23	44	.27	-.134	.027
23	2	22	41	.28	-.126	.042
24	2	20	58	.20	-.069	NS
25	3†	4	22	.35	-.052	NS
26	3	5	29	.37	-.034	NS
27	3†	5	34	.44	-.029	NS
28	3	14	29	.58	.268	.000

The mean values of MAPB en FTOE are presented. Correlation coefficients are based on the correlation between MABP and FTOE, sampled every 5 minutes.

† indicates infant who died.

measurements and may, therefore, not represent the entire study period. Additionally, infants with NEC received inotropes more often compared with infants without NEC. It was found that treated hypotension was associated with short- and long-time morbidity, which suggests an indirect relationship between inotropes and a pressure passive cerebral circulation.[27,28] Furthermore, a direct negative influence of dopamine on CAR has been reported.[29] Conversely, several studies demonstrated a protective role of inotropes by means of increasing CBF.[30-32]

Limitations of this study are the small size of the sample and the variation in the duration of the r_cSO_2 measurements for assessing CAR. These varied between 2 and 47 hours. Recent

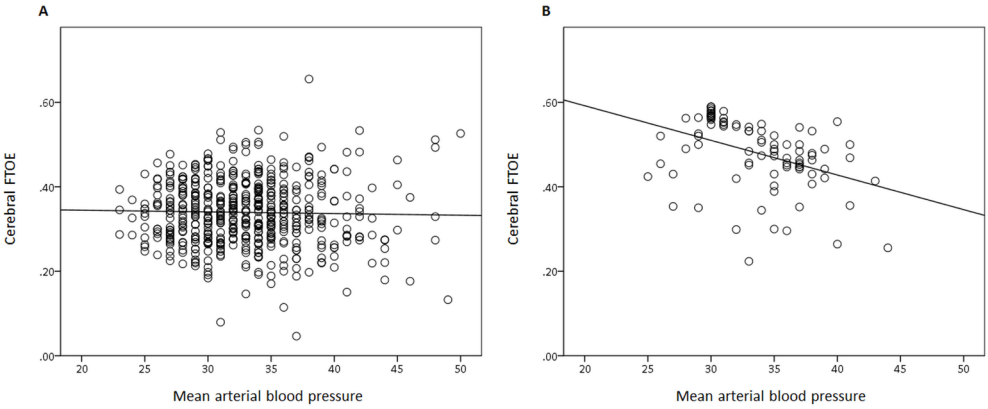

Figure 1. Scatter plot of cerebral FTOE and MABP values in an infant with adequate CAR (A) and an infant with impaired CAR (B).

research suggested that pressure-passivity can be transiently impaired.[18-21] In infants whom we measured for a short period of time we might, on the one hand, have measured an episode in which pressure-passivity was present or, on the other hand, an episode in which this was not the case. It is, therefore, possible that in such instances we either underestimated or overestimated the prevalence of impaired CAR. Furthermore, Verhagen *et al*. reported some limitations of the technique and of the method of analysis we used to assess CAR.[12] Even so, our data, as well as those of the study performed by Verhagen *et al.*, demonstrated that a considerable proportion of preterm infants can be identified as having impaired CAR by using the correlation between 5-minute measurements of cerebral FTOE and MABP.[12]

In conclusion, we demonstrated that 60% of preterm infants with NEC had a statistically significant negative correlation between cerebral FTOE and MABP, which may be indicative of impaired CAR. Our findings may imply that a pressure-passive CBF can be the underlying pathophysiological substrate for the development of white matter abnormalities and the long-term neurodevelopmental impairments in a substantial proportion of preterm infants with NEC. Furthermore, our results indicated that in those infants in whom NIRS measurements suggested impaired CAR, fluctuations in blood pressure could be potentially harmful. This warrants monitoring blood pressure closely as well as monitoring cerebral oxygenation by means of NIRS. Future studies need to be performed to elucidate when and how we need to intervene.

ACKNOWLEDGEMENTS

This study was part of the research program of the postgraduate school for Behavioural and Cognitive Neurosciences, University of Groningen. T.E. Schat and M.E. van der Laan were financially supported by a grant from the Junior Scientific Master Class of the University of Groningen.

We greatly acknowledge the help of Dr. T. van Wulfften Palthe for correcting the English manuscript.

REFERENCES

1. Sharma R, Hudak ML. A clinical perspective of necrotizing enterocolitis: Past, present, and future. *Clin Perinatol*. 2013;40(1):27-51.
2. Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA*. 2004;292(19):2357-2365.
3. Hintz SR, Kendrick DE, Stoll BJ, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. *Pediatrics*. 2005;115(3):696-703.
4. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. *Arch Dis Child Fetal Neonatal Ed*. 2007;92(3):F193-8.
5. Schulzke SM, Deshpande GC, Patole SK. Neurodevelopmental outcomes of very low-birth-weight infants with necrotizing enterocolitis: A systematic review of observational studies. *Arch Pediatr Adolesc Med*. 2007;161(6):583-590.
6. Martin CR, Dammann O, Allred EN, et al. Neurodevelopment of extremely preterm infants who had necrotizing enterocolitis with or without late bacteremia. *J Pediatr*. 2010;157(5):751-6.e1.
7. Roze E, Ta BD, van der Ree MH, et al. Functional impairments at school age of children with necrotizing enterocolitis or spontaneous intestinal perforation. *Pediatr Res*. 2011;70(6):619-625.
8. Shah DK, Doyle LW, Anderson PJ, et al. Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term. *J Pediatr*. 2008;153(2):170-5, 175.e1.
9. Volpe JJ. Postnatal sepsis, necrotizing entercolitis, and the critical role of systemic inflammation in white matter injury in premature infants. *J Pediatr*. 2008;153(2):160-163.
10. Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. *Arch Dis Child Fetal Neonatal Ed*. 2008;93(2):F153-61.
11. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011;364(3):255-264.
12. Verhagen EA, Hummel LA, Bos AF, Kooi EM. Near-infrared spectroscopy to detect absence of cerebrovascular autoregulation in preterm infants. *Clin Neurophysiol*. 2014;125(1):47-52.
13. Walsh MC, Kliegman RM. Necrotizing enterocolitis: Treatment based on staging criteria. *Pediatr Clin North Am*. 1986;33(1):179-201.
14. Brazy JE, Lewis DV, Mitnick MH, Jobsis vander Vliet FF. Noninvasive monitoring of cerebral oxygenation in preterm infants: Preliminary observations. *Pediatrics*. 1985;75(2):217-225.
15. Lemmers PM, Toet M, van Schelven LJ, van Bel F. Cerebral oxygenation and cerebral oxygen extraction in the preterm infant: The impact of respiratory distress syndrome. *Exp Brain Res*. 2006;173(3):458-467.
16. Naulaers G, Meyns B, Miserez M, et al. Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation. A validation study in piglets. *Neonatology*. 2007;92(2):120-126.
17. Schumann P, Touzani O, Young AR, Morello R, Baron JC, MacKenzie ET. Evaluation of the ratio of cerebral blood flow to cerebral blood volume as an index of local cerebral perfusion pressure. *Brain*. 1998;121 (Pt 7)(Pt 7):1369-1379.
18. Tsuji M, Saul JP, du Plessis A, et al. Cerebral intravascular oxygenation correlates with mean arterial pressure in critically ill premature infants. *Pediatrics*. 2000;106(4):625-632.
19. Soul JS, Hammer PE, Tsuji M, et al. Fluctuating pressure-passivity is common in the cerebral circulation of sick premature infants. *Pediatr Res*. 2007;61(4):467-473.
20. Wong FY, Leung TS, Austin T, et al. Impaired autoregulation in preterm infants identified by using spatially resolved spectroscopy. *Pediatrics*. 2008;121(3):e604-11.

21. Alderliesten T, Lemmers PM, Smarius JJ, van de Vosse RE, Baerts W, van Bel F. Cerebral oxygenation, extraction, and autoregulation in very preterm infants who develop peri-intraventricular hemorrhage. *J Pediatr*. 2013;162(4):698-704.e2.
22. Baerts W, van Bel F, Thewissen L, Derks JB, Lemmers PM. Tocolytic indomethacin: Effects on neonatal haemodynamics and cerebral autoregulation in the preterm newborn. *Arch Dis Child Fetal Neonatal Ed*. 2013;98(5):F419-23.
23. Ramaekers VT, Casaer P, Daniels H, Marchal G. The influence of blood transfusion on brain blood flow autoregulation among stable preterm infants. *Early Hum Dev*. 1992;30(3):211-220.
24. Vanderhaegen J, Vanhaesebrouck S, Vanhole C, Casaer P, Naulaers G. The effect of glycaemia on the cerebral oxygenation in very low birthweight infants as measured by near-infrared spectroscopy. *Adv Exp Med Biol*. 2010;662:461-466.
25. Pryds O, Greisen G, Lou H, Friis-Hansen B. Heterogeneity of cerebral vasoreactivity in preterm infants supported by mechanical ventilation. *J Pediatr*. 1989;115(4):638-645.
26. Kaiser JR, Gauss CH, Williams DK. The effects of hypercapnia on cerebral autoregulation in ventilated very low birth weight infants. *Pediatr Res*. 2005;58(5):931-935.
27. Fanaroff JM, Wilson-Costello DE, Newman NS, Montpetite MM, Fanaroff AA. Treated hypotension is associated with neonatal morbidity and hearing loss in extremely low birth weight infants. *Pediatrics*. 2006;117(4):1131-1135.
28. Kuint J, Barak M, Morag I, Maayan-Metzger A. Early treated hypotension and outcome in very low birth weight infants. *Neonatology*. 2009;95(4):311-316.
29. Eriksen VR, Hahn GH, Greisen G. Dopamine therapy is associated with impaired cerebral autoregulation in preterm infants. *Acta Paediatr*. 2014;103(12):1221-1226.
30. Seri I, Rudas G, Bors Z, Kanyicska B, Tulassay T. Effects of low-dose dopamine infusion on cardiovascular and renal functions, cerebral blood flow, and plasma catecholamine levels in sick preterm neonates. *Pediatr Res*. 1993;34(6):742-749.
31. Lundstrom K, Pryds O, Greisen G. The haemodynamic effects of dopamine and volume expansion in sick preterm infants. *Early Hum Dev*. 2000;57(2):157-163.
32. Munro MJ, Walker AM, Barfield CP. Hypotensive extremely low birth weight infants have reduced cerebral blood flow. *Pediatrics*. 2004;114(6):1591-1596.

CHAPTER 5

CEREBRAL AND INTESTINAL OXYGENATION IN RELATION TO THE DEVELOPMENT OF NECROTIZING ENTEROCOLITIS

Trijntje E. Schat, Anne G.J.F. van Zoonen, Michelle E. van der Laan, Arend F. Bos, Christian V. Hulzebos, H. Marike Boezen, Jan B.F. Hulscher, Elisabeth M.W. Kooi

Submitted

ABSTRACT

Objectives: To investigate whether cerebral and intestinal regional tissue oxygen saturation (rSO_2) and fractional tissue oxygen extraction (FTOE) values predict the onset of necrotizing enterocolitis (NEC).

Methods: A prospective case-control study. Infants with gestational age (GA) < 32 weeks and birth weight (BW) < 1200 grams were included. For every NEC case we matched two controls based on GA, BW, and presence of a hemodynamically significant patent ductus arteriosus. Cerebral and intestinal oxygenation were monitored two hours daily during the first five days after birth and once a week thereafter until five weeks after birth or until NEC developed. Kaplan-Meier analyses were used to assess the ability of near-infrared spectroscopy (NIRS) measurements to predict the development of NEC.

Results: We included ten infants (median (range) GA 27.1 (24.6-29.4) weeks, BW 903 (560-1630) grams) who developed NEC at median postnatal day 13 (range, 4-43 days), and 20 matched controls. Infants with cerebral rSO_2 < 70% in the first 48 hours after birth developed NEC significantly more often than infants with cerebral rSO_2 ≥ 70% (P = .005). Intestinal FTOE was higher in infants who developed NEC compared with controls during the last NIRS measurement before NEC onset (median 0.65 vs. 0.44, P = 0.04), which was performed at a median of 2 (range, 1-7) days prior to NEC development.

Conclusions: Cerebral NIRS monitoring early after birth might be valuable to predict the onset of NEC. Additionally, our results suggest that intestinal perfusion is impaired before the onset of clinical NEC.

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most severe gastrointestinal disease in preterm infants, with mortality rates up to 40%.[1] Short- and long-term gastrointestinal and neurodevelopmental impairment occurs frequently.[2-5] Preventing development of NEC is currently considered the best strategy to minimize these devastating short- and long-term consequences.[6]

Near-infrared spectroscopy (NIRS) measures regional tissue oxygen saturation (rSO_2) non-invasively. When transcutaneous arterial oxygen saturation (SpO_2) is obtained simultaneously, fractional tissue oxygen extraction (FTOE) can be calculated.[7] FTOE is thought to reflect the balance between oxygen delivery and oxygen consumption, and might therefore be used as an early indicator of inadequate tissue perfusion.[7] As bowel ischemia is hypothesized to play a role in NEC development, NIRS could be a helpful tool for predicting the onset of NEC in preterm infants.[8,9] Patel *et al.* found lower intestinal rSO_2 ($r_{int}SO_2$) values in the first week of life in preterm infants who later developed NEC compared to infants who did not develop NEC.[10] Additionally, it was found that $r_{int}SO_2$ was lower and showed less variability just before NEC onset, when symptoms were not yet present.[11]

Measurements of cerebral rSO_2 (r_cSO_2) might provide additional information concerning the underlying mechanism responsible for NEC development, since impaired intestinal perfusion might be the result of a compromised systemic circulation. Simultaneous measurements of cerebral and intestinal rSO_2 values might help to determine whether this is indeed the case. We therefore investigated the possibility to use cerebral and intestinal NIRS measurements in the first days after birth to differentiate high-risk infants who went on to develop NEC from those who did not. Furthermore, we compared cerebral and intestinal rSO_2 and FTOE values of the last measurement before the onset of NEC between infants who developed NEC and those who did not.

METHODS

Patient population

We performed a case-control study. Patients and controls were derived from a prospective observational cohort study performed at the neonatal intensive care unit (NICU) of University Medical Center Groningen between October 2012 and February 2014. The study was registered in the Dutch Trial Registry under number NTR4153. In the large cohort study we included 100 consecutive preterm infants at high risk for developing NEC. High risk was defined as being born at a gestational age (GA) of less than 30 weeks, with a birth weight (BW) below 1000 grams, at a GA of less than 32 weeks with a BW below 1200 grams, the presence of cardiac disease leading to impaired intestinal blood flow, or being born from a mother who received indomethacin for tocolysis. Infants with abdominal wall defects and infants who could not be measured with NIRS within 72 hours after birth were excluded.

We obtained written informed parental consent in all cases. The study was approved by the institutional ethics review board of University Medical Center Groningen.

For the present case-control study, we selected all NEC cases and matched two controls to each infant who developed NEC, using the following criteria, in descending order of importance: GA, BW, and the presence of a hemodynamically significant patent ductus arteriosus (PDA).

NEC diagnosis

NEC was diagnosed by the attending radiologist when pneumatosis intestinalis, portal venous gas, or both were present on abdominal radiographic examination.[12] Afterwards, five consultants classified the infants according to modified Bell's stages.[12] Consensus was reached in all cases.

Near-infrared spectroscopy

We used the INVOS 5100C monitor (Covidien, Mansfield, MA, USA) with neonatal SomaSensors (Covidien) to measure r_cSO_2 and $r_{int}SO_2$. We placed the sensor on the left or right frontoparietal side of the head to measure r_cSO_2 and centrally just below the umbilicus to measure $r_{int}SO_2$. Sensors were kept in place using Mepitel (Mölnlycke, Sweden) or an elastic bandage.
We performed NIRS measurements two hours daily, from day one to five after birth, and weekly thereafter until the fifth week (day 36 after birth), until the infant was discharged from the NICU, or until NEC developed, whichever came first.
The first NIRS measurement was classified into either day one (< 24 hours after birth) or day two (> 24 hours < 48 hours after birth). Timing of sequential NIRS measurements was based on the date of the first NIRS measurement.

Clinical variables

We collected the following clinical data from patient reports: GA, BW, gender, postnatal age at time of NEC onset, multiple gestations, the administration of antenatal steroids, cause of prematurity, Apgar scores at one and five minutes, administration of antibiotics in the first 48 hours after birth, continuation of antibiotics more than 48 hours after birth, mortality, and length of NICU stay. Furthermore, we documented ventilator use, presence of a hemodynamically significant PDA, and use of medication (inotropes, red blood cell transfusions, volume expansion, ibuprofen).

Data analysis and statistical analysis

We collected cerebral and intestinal rSO_2 values once every 6 seconds and SpO_2 once every 5 seconds. We then matched SpO_2 values that corresponded temporally to the rSO_2 values. Next, FTOE values were calculated for the cerebral and intestinal region separately, using the synchronized rSO_2 and SpO_2 values. We allowed the first 10 minutes of each NIRS measurement for stabilization. As a result, 110 minutes of available data could be used to calculate the daily mean values of r_cSO_2, $r_{int}SO_2$, cFTOE, and intFTOE.

First, we determined whether r_cSO_2, $r_{int}SO_2$, cFTOE and/or intFTOE values predicted the onset of NEC. We therefore used the first measurement after birth, which was obtained on either day one or two. Since it was found that low rSO_2 values corresponded to the onset of NEC,[10,11,13] we chose to use the 25th percentile for r_cSO_2 and $r_{int}SO_2$ values and the 75th percentile for cFTOE and intFTOE values as a cut-off. We compared infants with r_cSO_2 and $r_{int}SO_2$ values below the 25th percentile to infants with r_cSO_2 and $r_{int}SO_2$ values above the 25th percentile. Similarly, we compared infants with cFTOE and intFTOE values below the 75th percentile to infants with cFTOE and intFTOE values above the 75th percentile. We performed Kaplan-Meier analyses to determine whether the occurrence of NEC was significantly different between the aforementioned groups. We used the log-rank test to determine if there were significant differences in the Kaplan-Meier plots for the different groups: low versus high rSO_2 and FTOE values. Then, we performed a logistic regression analysis for those variables that had significantly different Kaplan-Meier curves and calculated odds ratios.

Next, we determined the course of cerebral and intestinal rSO_2 and FTOE in individual infants. We used the Mann-Whitney test to assess differences in median values of the last cerebral and intestinal rSO_2 and FTOE values before NEC development between preterm infants who developed NEC and infants who did not.

Finally, we determined the variability of cerebral and intestinal rSO_2 measurements of the first NIRS measurement after birth and of the last NIRS measurement prior to NEC development. For this purpose, we divided the 110 minutes of NIRS data that were available for each day in eleven blocks of 10-min and subsequently calculated means of r_cSO_2 and $r_{int}SO_2$ values for every 10-min block. We compared each 10-min mean with the infant's daily mean and determined the amount of 10-min means that were 15% below or under the daily mean.[14] We used the Mann-Whitney test to determine whether there were significant differences in variability between the two groups during the first NIRS measurements after birth, and during the last NIRS measurements prior to the development of NEC.

In order to test differences in clinical parameters between infants who developed NEC and infants who did not, we used the chi-square test or Fischer exact test for categorical data and the Mann-Whitney test for continuous data. We used SPSS 22.0 software for Windows (IBM SPSS Statistics 22, IBM Corp., Armonk, New York, USA) for statistical analyses. We considered a *P* value < .05 to be statistically significant.

RESULTS

Of the 99 infants that were included in the study protocol, 11 preterm infants developed NEC of whom 10 infants with available NIRS measurements (Figure 1). From the remaining 88 included infants, 20 were matched to the 10 preterm infants with NEC and served as a control group. Of the ten infants with NEC, two infants were eventually classified as Bell's stage 2 and eight infants as Bell's stage 3 of whom six infants developed a bowel perforation.

None of the infants with NEC nor any of the control infants had cardiac diseases or had received indomethacin antenatally.

Median postnatal age at time of NEC onset was 13 (range, 4-43) days. In preterm infants who developed NEC, we were able to calculate mean r_cSO_2 values for 62/64 (97%) 2-hour periods and mean $r_{int}SO_2$ values for 20/64 (31%) 2-hour periods. In preterm infants who did not develop NEC, we were able to calculate mean r_cSO_2 values for 122/128 (95%) 2-hour periods and mean $r_{int}SO_2$ values for 41/128 (32%) 2-hour periods. Placing the infraumbilical sensor was often not possible due to the presence of an umbilicous venous catheter taped to the infraumbilical skin and/or due to lack of space in very low birth weight and small for gestational age infants.

We present clinical characteristics of the study population in Table 1. Apart from significantly higher mortality rates in preterm infants with NEC compared to controls, no other differences were observed.

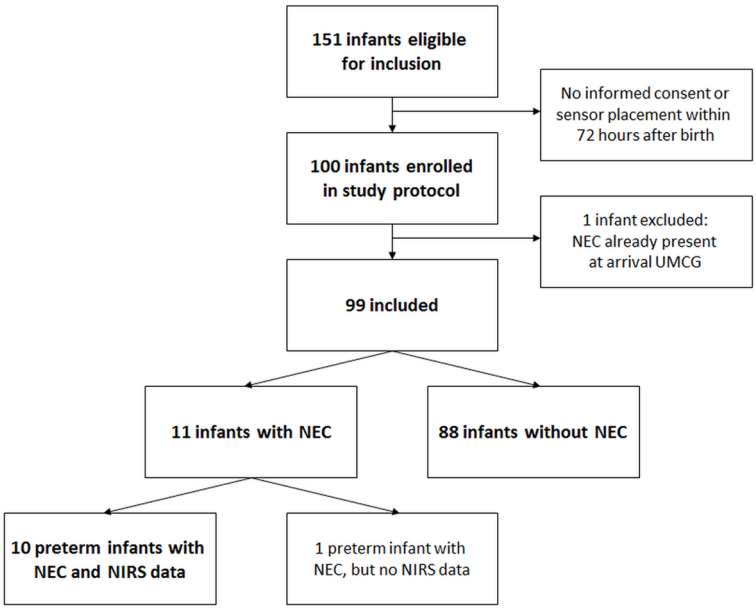

Figure 1. Flow diagram of the study.

Cerebral and intestinal rSO_2 and FTOE values in the first days after birth and the development of NEC

In 21/30 patients (70%) the first NIRS measurement was performed within 24 hours (day one) after birth, and in 9/30 (30%) between 24 hours and 48 hours (day two) after birth. All infants were monitored in the cerebral region (30/30: 100%) and seven infants also in the intestinal region (7/30: 23%). SpO_2 values were not significantly different between infants

Table 1. Patient characteristics of preterm infants with NEC and their matched controls.

	NEC cases (n = 10)	Controls (n = 20)	*P* value
Gestational age (weeks)	27.1 (24.6-29.4)	27.6 (25-29.7)	.58
Birth weight (grams)	903 (560-1630)	960 (615-1330)	.88
Male:Female	6:4	10:10	.71
Multiple gestations	3 (30)	4 (20)	.66
Antenatal steroids	9 (90)	18 (90)	.99
Reason prematurity			
PPROM (>24h)	3 (30)	3 (15)	.37
Spontaneous premature birth	3 (30)	10 (50)	.44
Induced birth – maternal	2 (20)	2 (10)	.58
Induced birth – fetal	2 (20)	4 (20)	.99
Induced birth – maternal and fetal	-	1 (5)	.99
Apgar score 1 min	4 (1-7)	5 (2-10)	.18
Apgar score 5 min	7 (1-9)	7 (3-10)	.74
Antibiotics < 48 hours after birth	8 (80)	17 (85)	.99
Antibiotics > 48 hours after birth	7 (70)	13 (65)	.99
Hemodynamically significant PDA	5 (50)	8 (40)	.71
Ibuprofen	5 (50)	8 (40)	.71
Mechanical ventilation	7 (70)	16 (80)	.65
RBC transfusion	8 (80)	13 (65)	.68
RBC transfusion in 48 hours before NEC onset	3 (38)		
Fluid resuscitation	6 (60)	8 (40)	.44
Inotropes	4 (40)	3 (15)	.18
Length NICU stay (days)	46 (6-89)	39 (9-103)	.73
Death	4 (40)	1 (5)	.03

Data are expressed as median (range) or as number (percentage) unless specified otherwise.

Abbreviations: NEC - necrotizing enterocolitis; NICU - neonatal intensive care unit; PDA - patent ductus arteriosus; PPROM - preterm premature rupture of membranes; RBC - red blood cell.

who developed NEC compared to infants who did not (median: 91% (range, 48-99%) versus 90% (range, 84-97%), *P* = .69).

The values of the 25th percentile of r_cSO_2 and $r_{int}SO_2$ were 70% and 30%, respectively. The values of the 75th percentile of cFTOE and intFTOE were .23 and .65, respectively.

Infants with r_cSO_2 values below the 25th percentile (n=7) developed NEC significantly more often in the first 43 days after birth than infants with r_cSO_2 values above the 25th percentile (n=23), *P* = .005 (Figure 2). The occurrence of NEC was not significantly different between infants with $r_{int}SO_2$ values below and above the 25th percentile, or between infants with cFTOE and intFTOE values below and above the 75th percentile.

Using logistic regression analysis, we found that the risk of NEC increased with an odds ratio of 9.00 (95% CI 1.33-61.14, *P* = .03) when infants had r_cSO_2 values < 70%.

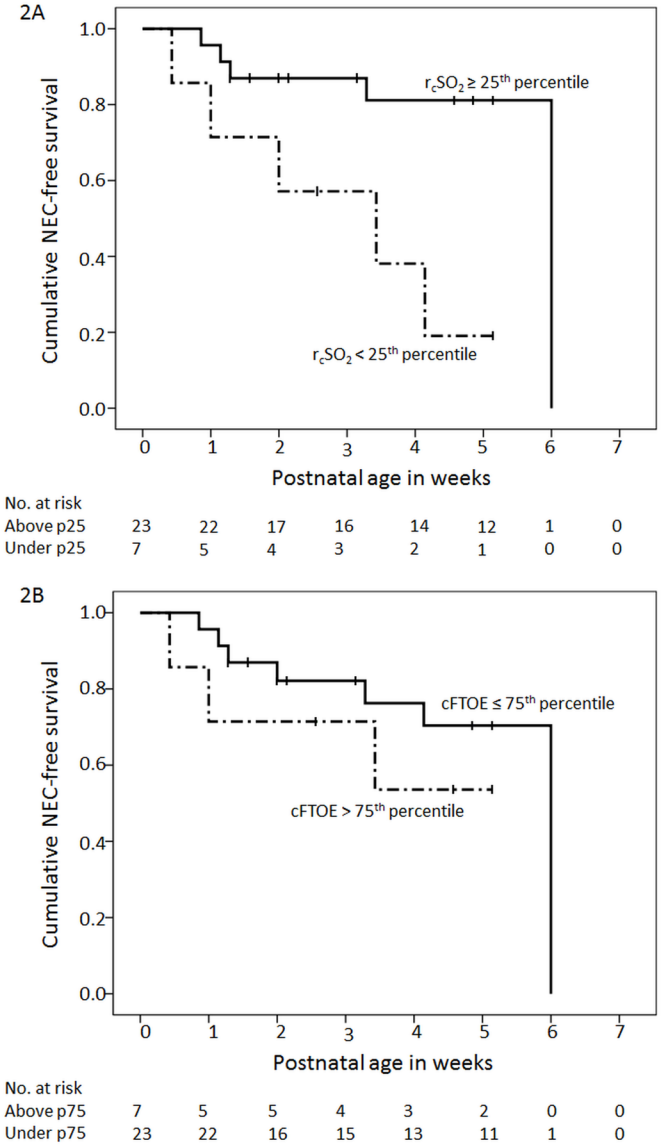

Figure 2. Kaplan-Meier plots illustrating the occurrence of NEC in infants with r_cSO_2 values below (━ • ━) and above (━━━) the 25th percentile (A) and infants with cFTOE values below (━━━) and above (━ • ━) the 75th percentile (B) on the first measurement on day one or two.

Cerebral and intestinal rSO_2 and FTOE values the week prior to NEC onset

The course of cerebral and intestinal rSO_2 and FTOE in individual infants who developed NEC did not reveal any specific patterns. No significant differences of these values were found between the last and the penultimate measurement before NEC.

The last NIRS measurement before NEC development was obtained at a median of 2 (range, 1-7) days prior to NEC development; median postnatal day was 8 (range, 3-36) days. SpO_2 values were not significantly different between infants who developed NEC and infants who did not (median 92% (range: 86-100%) versus 90% (range: 84-99%), *P* = .16). We found significantly higher intFTOE values in preterm infants who developed NEC than in infants who did not develop NEC (median 0.65 (range, 0.49-0.84) versus 0.44 (range, 0.17-0.70), *P* = .04, Figure 3D). Furthermore, $r_{int}SO_2$ tended to be lower and cFTOE values higher in infants who developed NEC compared to controls ($r_{int}SO_2$, median 33% versus 48%, *P* = .09, Figure 3B; cFTOE, median 0.36 versus 0.24, *P* = .08, Figure 3C).

Variability

We did not find significant differences for the variability between infants with and infants without NEC in the first NIRS measurement after birth (r_cSO_2, median (range) 0% (0%-0%) versus 0% (0%-33%), *P* = .16; $r_{int}SO_2$, median 0% (0%-18%) versus 5% (0%-9%), *P* = .63), nor in the last NIRS measurement prior to NEC onset (r_cSO_2, median 0% (0%-3%) versus 0% (0%-18%), *P* = .24; $r_{int}SO_2$, median 0% (0%-18%) versus 0% (0%-0%), *P* = .13).

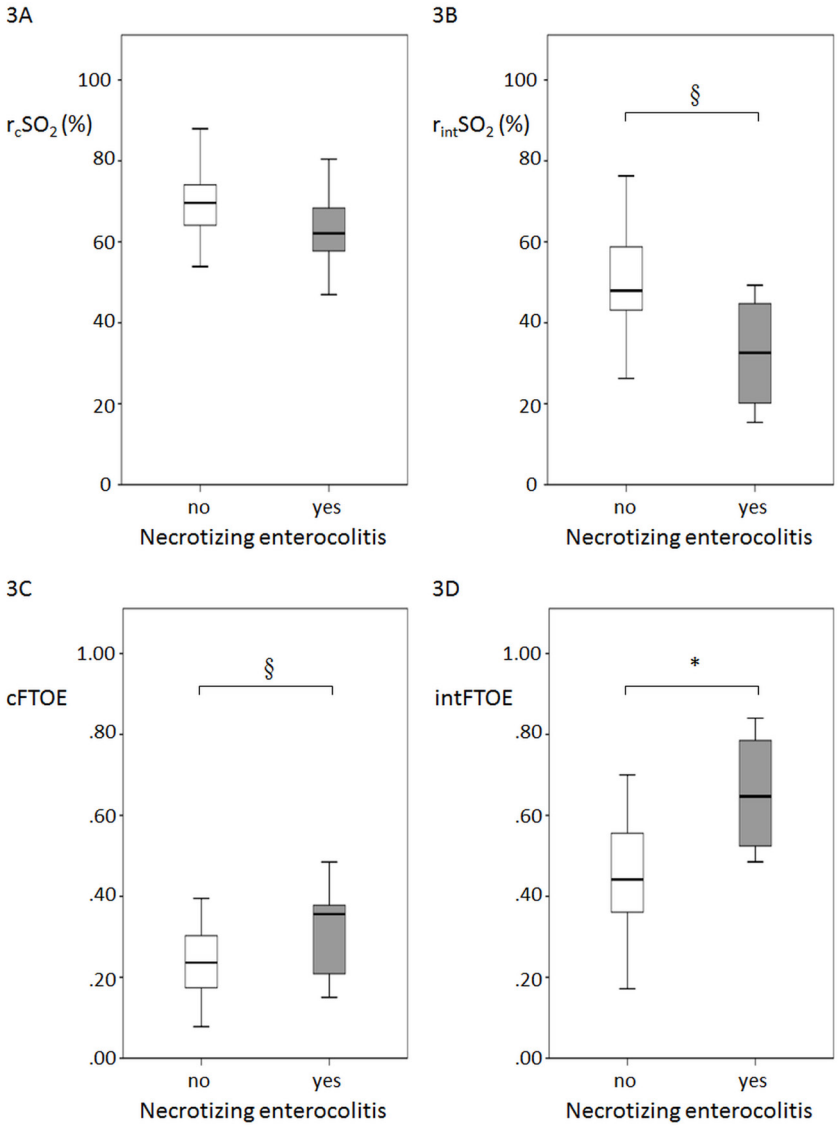

Figure 3. Median values of r_cSO_2 (A), $r_{int}SO_2$ (B), cFTOE (C), and intFTOE (D) of the last NIRS measurement prior to NEC onset in infants who developed NEC and their controls. Data are shown in box and whisker plots. Dots represent outliers.

* indicates $P < .05$ and § $P < .10$.

DISCUSSION

In this study we found that r_cSO_2 values might be useful to predict the onset of NEC in preterm infants. The probability of developing NEC was nine-fold higher in infants with r_cSO_2 values < 70% in the first two days after birth compared with infants with r_cSO_2 values ≥ 70%. Furthermore, we found significantly higher intestinal FTOE values in the week prior to NEC development in preterm infants who developed NEC compared with matched controls. Variability did not differ between preterm infants who went on to develop NEC and infants who did not.

Our results suggest that monitoring r_cSO_2 might be helpful in identifying infants who will develop NEC. We found that infants with r_cSO_2 values < 70% in the first 48 hours after birth had a nine-fold higher risk of developing NEC. This finding suggests that infants who go on to develop NEC have impaired cerebral oxygenation as early as within 48 hours after birth. We offer several explanations for this finding. First, low r_cSO_2 values might be caused by low SpO_2 values as it was recently found that low SpO_2 values were associated with NEC development.[15] However, we did not find lower SpO_2 values in infants who developed NEC compared to controls (median 91% versus 90%, P = .69). Nonetheless, since we only measured two hours daily, we might have missed episodes in which infants were in the lower SpO_2 range. Secondly, low r_cSO_2 values might be the result of a low systemic perfusion. Because several clinical factors such as ventilatory status and presence of PDA were not different between cases and controls, we believe that lower r_cSO_2 values cannot be explained by these factors. Still, infants who go on to develop NEC may be relatively underperfused at birth due to variables we did not investigate, such as inflammation, maternal medication, and/or a more pronounced left to right flow across the PDA. The inciting event or chain of events that lead to development of NEC may therefore be set off before, during and/or just after birth. Further research in a larger patient population is warranted to investigate this hypothesis.

As opposed to r_cSO_2 values, $r_{int}SO_2$ values within the first days after birth were not associated with NEC development later on. We were, however, only able to measure $r_{int}SO_2$ in seven (7/30, 23%) preterm infants. In the very preterm and small for gestational age infants space was lacking for adequate sensor placement. The presence of an umbilicous venous catheter taped to the skin hindered sensor placement as well. The resultant small sample size may have limited our ability to detect significant differences in intestinal oxygenation values between the two groups. However, our experience indicates that monitoring at the infraumbilical region is accompanied with practical difficulties in preterm infants in the first days after birth, limiting the usefulness of this measurement procedure in clinical practice.

Patel *et al.* recently demonstrated that $r_{int}SO_2$ values were lower in the first week after birth in infants who later developed NEC compared with infants who did not.[10] We did not find such a difference, perhaps because of the small sample size of this study. Differences between study designs might also explain the dissimilar findings. In the study of Patel *et al.* infants with NEC were of lower GAs and had lower BWs than their controls,[10] whilst our cases

were comparable with controls regarding GA and BW. It has been reported that intestinal rSO_2 measurements might be dependent on GA.[14] Furthermore, Patel *et al.* measured daily for five minutes,[10] whilst we measured continuously for two hours every day. All other factors being equal, a more robust measurement should have made our chance of finding a difference greater rather than smaller.

With regard to the last NIRS measurement prior to NEC onset, we found approximately one and a half times higher intFTOE values in preterm infants with NEC than in controls. This finding supports the hypothesis that infants who develop NEC have decreased intestinal perfusion prior to the development of NEC.[8,9] As a predictor of NEC in individual infants this measurement is not clinically useful, because of the large intra-individual range of the intFTOE values. However, it provides insight into the role of intestinal perfusion in the pathophysiology of NEC. Further studies are needed to investigate the possibility of using intestinal oxygenation values to predict the onset of NEC.

With regard to the variability measurements, we did not find significant differences between infants with NEC and their matched controls. Cortez *et al.* suggested that loss of variability pointed towards the development of NEC.[11] Possibly, the method we used to determine the variability might not be sensitive enough to detect subtle changes. Further research is warranted to assess the usefulness of using variability for detecting an infant at risk for developing NEC.

In order to be able to adequately interpret our results, we have to keep in mind that the current NIRS technology has limitations. Since there are different NIRS devices, each with their own algorithm, our results are only representative for the INVOS 5100C device.[16-18] Moreover, peristalsis, gut movements, air, and stools complicate the interpretation of intestinal NIRS measurements.[14,19,20]

The strength of this study is that we matched the infants who developed NEC to controls, based on GA, BW, and presence of hemodynamically significant PDA. Moreover, we measured two hours daily. A limitation is that we were not able to apply the infraumbilical sensor in every infant due to lack of space or the presence of an umbilical catheter. As a result, we were unable to obtain $r_{int}SO_2$ values in the smallest infants, which could have biased our results.

CONCLUSIONS

Cerebral rSO_2 values in the first two days after birth are predictive for NEC in preterm infants with a GA of less than 32 weeks. Although impaired intestinal perfusion is possibly present before the onset of NEC, the usefulness of monitoring intestinal oxygenation values to predict the onset of NEC needs to be investigated further.

ACKNOWLEDGEMENTS

This study was part of the research program of the postgraduate school for Behavioural and Cognitive Neurosciences, University of Groningen. T.E. Schat, A.G.J.F. van Zoonen, and M.E.

van der Laan were financially supported by a grant from the Junior Scientific Master Class of the University of Groningen.
We greatly acknowledge the help of Prof. dr. A.E.J. Dubois for correcting the English manuscript.

REFERENCES

1. Fitzgibbons SC, Ching Y, Yu D, et al. Mortality of necrotizing enterocolitis expressed by birth weight categories. *J Pediatr Surg*. 2009;44(6):1072-1075;discussion 1075-1076.
2. Hintz SR, Kendrick DE, Stoll BJ, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. *Pediatrics*. 2005;115(3):696-703.
3. Schulzke SM, Deshpande GC, Patole SK. Neurodevelopmental outcomes of very low-birth-weight infants with necrotizing enterocolitis: A systematic review of observational studies. *Arch Pediatr Adolesc Med*. 2007;161(6):583-590.
4. Roze E, Ta BD, van der Ree MH, et al. Functional impairments at school age of children with necrotizing enterocolitis or spontaneous intestinal perforation. *Pediatr Res*. 2011;70(6):619-625.
5. Murthy K, Yanowitz TD, DiGeronimo R, et al. Short-term outcomes for preterm infants with surgical necrotizing enterocolitis. *J Perinatol*. 2014;34(10):736-740.
6. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011;364(3):255-264.
7. Naulaers G, Meyns B, Miserez M, et al. Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation. A validation study in piglets. *Neonatology*. 2007;92(2):120-126.
8. Nowicki PT. Ischemia and necrotizing enterocolitis: Where, when, and how. *Semin Pediatr Surg*. 2005;14(3):152-158.
9. Watkins DJ, Besner GE. The role of the intestinal microcirculation in necrotizing enterocolitis. *Semin Pediatr Surg*. 2013;22(2):83-87.
10. Patel AK, Lazar DA, Burrin DG, et al. Abdominal near-infrared spectroscopy measurements are lower in preterm infants at risk for necrotizing enterocolitis. *Pediatr Crit Care Med*. 2014;15(8):735-741.
11. Cortez J, Gupta M, Amaram A, Pizzino J, Sawhney M, Sood BG. Noninvasive evaluation of splanchnic tissue oxygenation using near-infrared spectroscopy in preterm neonates.*J Matern Fetal Neonatal Med*. 2011;24(4):574-582.
12. Walsh MC, Kliegman RM. Necrotizing enterocolitis: Treatment based on staging criteria. *Pediatr Clin North Am*. 1986;33(1):179-201.
13. Gay AN, Lazar DA, Stoll B, et al. Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. *J Pediatr Surg*. 2011;46(6):1034-1040.
14. McNeill S, Gatenby JC, McElroy S, Engelhardt B. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. *J Perinatol*. 2011;31(1):51-57.
15. Lakshminrusimha S, Manja V, Mathew B, Suresh GK. Oxygen targeting in preterm infants: A physiological interpretation. *J Perinatol*. 2015;35(1):8-15.
16. Matcher SJ, Elwell CE, Cooper CE, Cope M, Delpy DT. Performance comparison of several published tissue near-infrared spectroscopy algorithms. *Anal Biochem*. 1995;227(1):54-68.
17. Underwood MA, Milstein JM, Sherman MP. Near-infrared spectroscopy as a screening tool for patent ductus arteriosus in extremely low birth weight infants. *Neonatology*. 2007;91(2):134-139.
18. Dix LM, van Bel F, Baerts W, Lemmers PM. Comparing near-infrared spectroscopy devices and their sensors for monitoring regional cerebral oxygen saturation in the neonate.*Pediatr Res*. 2013;74(5):557-563.
19. Teller J, Wolf M, Keel M, Bucher HU, Fanconi S, Baenziger O. Can near infrared spectroscopy of the liver monitor tissue oxygenation? *Eur J Pediatr*. 2000;159(7):549.

20. Said MM, Niforatos N, Rais-Bahrami K. Validation of near infrared spectroscopy to measure abdominal somatic tissue oxygen saturation in neonates. *J Neonatal Perinatal Med*. 2013;6(1):23-30.

CHAPTER 6

USING NEAR-INFRARED SPECTROSCOPY TO PREDICT THE DEVELOPMENT OF NECROTIZING ENTEROCOLITIS

Trijntje E. Schat, Maarten Schurink, Michelle E. van der Laan, Jan B.F. Hulscher, Christian V. Hulzebos, Arend F. Bos, Elisabeth M.W. Kooi

Submitted

ABSTRACT

Objectives: To investigate whether cerebral, liver, and infraumbilical regional tissue oxygen saturation (rSO_2) and fractional tissue oxygen extraction (FTOE) could be used to diagnose necrotizing enterocolitis (NEC) and complicated NEC (Bell's stage 3B or death) during its early stages.

Methods: A prospective observational cohort study of preterm infants with suspected or diagnosed NEC. We compared mean 8-hour cerebral, liver, and infraumbilical rSO_2 and FTOE values of infants with suspected and definite NEC and of infants with uncomplicated and complicated NEC in the first 48 hours after onset of NEC symptoms. Furthermore, we determined cut-off values by generating receiver operating characteristics curves in case of significant differences in the first 8-hour mean values of rSO_2 between infants with suspected and definite NEC and between infants with uncomplicated and complicated NEC.

Results: We included 33 patients: 13 without NEC, 10 with uncomplicated NEC, and 10 with complicated NEC. We found no significant differences in the first 24 hours after onset of NEC symptoms in rSO_2 and FTOE values between infants with suspected and definite NEC. In preterm infants with complicated NEC, we observed significantly lower cerebral, liver, and infraumbilical rSO_2 values and higher FTOE values within 24 hours after onset of NEC symptoms in comparison to infants with uncomplicated NEC. A continuous cerebral $rSO_2 \leq 71\%$ and liver $rSO_2 \leq 59\%$ in the first 8 hours after onset of NEC symptoms predicted the onset of complicated NEC with a sensitivity of 1.00 and specificity of 0.80, and a sensitivity of 1.00 and specificity of 1.00, respectively.

Conclusions: By measuring cerebral and splanchnic oxygenation it may be possible to differentiate complicated NEC in preterm infants from uncomplicated NEC. NIRS monitoring did not proof useful for distinguishing between suspected and definite NEC.

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most devastating gastrointestinal disease in the neonatal intensive care unit. It is associated with detrimental short-term and long-term outcomes, including high mortality rates and impaired neurodevelopmental outcome.[1,2] Currently, we lack the tools and tests to reliably diagnose NEC in its early stage and to predict its progression to becoming a complicated disease (i.e. perforated bowel corresponding to Bell's stage 3B, or death).

Near-infrared spectroscopy (NIRS) might be a useful bedside tool to diagnose the earliest stages of NEC. In previous studies it was found that NIRS measurements differed between preterm infants with and without bowel ischemia.[3-5] NIRS measures regional tissue oxygen saturation (rSO_2) non-invasively. Using simultaneous measurements of transcutaneous arterial oxygen saturation (SpO_2), fractional tissue oxygen extraction (FTOE) can be calculated.[6] It reflects the balance between oxygen supply and consumption in tissue and can, therefore, be used as an indicator of inadequate tissue perfusion and oxygenation.[6] Since bowel ischemia seems to be strongly associated with the development of NEC, complicated NEC in particular,[7] measuring splanchnic tissue oxygen saturation and extraction might help the clinician to diagnose NEC from its earliest stage onward.

Our first aim was to investigate the diagnostic value of splanchnic NIRS monitoring to discriminate between definite NEC (Bell's stages 2 & 3) and suspected NEC (Bell's stage 1) at the onset of the disease. Our second aim was to determine whether splanchnic NIRS monitoring could be used to discriminate between infants with NEC that would develop without complications (uncomplicated NEC) and infants with NEC that would develop with complications (complicated NEC). The latter was defined as the infant developing a bowel perforation requiring surgery (Bell's stage 3B), or death. We hypothesized that as a result of hypoxic and/or necrotic intestinal tissue splanchnic rSO_2 values would be lower and splanchnic FTOE values would be higher in preterm infants who developed (complicated) NEC.

METHODS

Patient population

Between October 2010 and October 2012 we conducted a prospective observational cohort study in the neonatal intensive care unit of University Medical Center Groningen, a tertiary referral center. The study was registered in the Dutch Trial Registry under number NTR3239. We included preterm infants without abdominal wall defects who were suspected of having NEC or who had already been diagnosed with NEC. Abdominal radiographs were made as soon as possible after suspicion of NEC; the diagnosis was confirmed if pneumatosis intestinalis was present. The modified Bell's staging criteria were used for diagnosis.[8] In case of definite NEC (minimal Bell's stage 2), our protocol indicates that sequential abdominal radiographs be taken every 8 to 12 hours until it is evident that radiographic signs of NEC have resolved and clinical signs have stabilized.
Written informed parental consent was obtained in all cases. The study was approved by the institutional ethics review board of University Medical Center Groningen.

Near-infrared spectroscopy

We used the INVOS 5100C near-infrared spectrometer (Covidien, Mansfield, MA, USA) in combination with the neonatal SomaSensors (Covidien) to measure oxygen saturation values of cerebral tissue and in the splanchnic region. We placed the SomaSensors to the left or right frontoparietal side of the infant's head to measure cerebral tissue oxygen saturation (r_cSO_2). The oxygen saturation of the splanchnic region was measured at two abdominal locations: below the right costal arch to measure liver tissue oxygen saturation ($r_{liv}SO_2$), and infraumbilically on the central abdominal wall to measure intestinal tissue oxygen saturation ($r_{int}SO_2$). The sensors were held in place by elastic bandaging or Mepitel (Mölnlycke, Sweden) and were only removed temporarily during routine nursing care, clinical assessments, and radiographic examinations. Afterwards, they were replaced onto the same location. NIRS monitoring started as soon as possible after NEC was suspected or diagnosed and was continued for 48 hours. Simultaneously, we measured SpO_2 and calculated FTOE with the equation: $FTOE = (SpO_2 – rSO_2) / SpO_2$.[6]
We previously reported on the feasibility and safety of monitoring oxygenation in both the liver and infraumbilical region and the correlation and agreement between these measurements.[9] The infants reported in that article are also part of the study group described in this manuscript. However, we did not report any findings concerning the course of rSO_2 and FTOE values in relation to the development of definite NEC and complicated NEC.

Clinical variables

Prospectively, we collected neonatal characteristics including gestational age, postnatal age at first NIRS measurement, birth weight, and gender. Furthermore, we documented the presence or absence of anemia (defined as a hemoglobin level < 8.0 mmol/L),

thrombocytopenia (defined as a platelet count < 150 x 10^9/L), and metabolic acidosis (defined as pH < 7.30 and HCO_3^- < 22 mmol/L) within 24 hours before and 24 hours after onset of NEC symptoms. Furthermore, we registered signs of circulatory failure and patency of the ductus arteriosus (determined by echocardiography) during the first 48 hours after onset of NEC symptoms, and treatment for a patent ductus arteriosus before the onset of NEC symptoms. Circulatory failure was defined as hemodynamic instability and scored by the need for volume expansion or the use of inotropes or both, from one hour before onset of NEC symptoms until the first 48 hours after onset, or until surgery, whichever came first.
Onset of NEC symptoms was defined as the time of the first abdominal radiographic examination after clinical suspicion of NEC, including the radiographs done in the referring hospitals. After completion of the study a panel of four experts (MS, JBFH, AFB, EMWK), blinded for the NIRS measurements, initially classified the infants independently of one another into onset and end-stage modified Bell's stages using clinical and radiological parameters. For those infants who had been classified differently by the individual panel members the final Bell's stage was determined by consensus.
To address our first aim, we classified the infants into two groups: infants with suspected NEC (Bell's stage 1) and infants with definite NEC (Bell's stages 2 & 3). For our second aim, we analyzed the differences between infants with uncomplicated NEC and infants with complicated NEC (Bell's stage 3B, or death as a consequence of NEC).

Statistical analysis

We used SPSS 22.0 software for Windows (IBM SPSS Statistics 22, IBM Corp., Armonk, New York, USA) for the statistical analyses.
R_cSO_2, $r_{liv}SO_2$, and $r_{int}SO_2$ values were recorded by the INVOS 5100C every 6 seconds. SpO_2 was registered every 5 minutes. We then matched one rSO_2 value that corresponded time wise to every SpO_2 value, leaving one measurement per 5 minutes for rSO_2 and SpO_2. Next, we constructed six 8-hour periods starting from onset of NEC symptoms (first abdominal radiographic examination) and calculated 8-hour means of r_cSO_2, $r_{liv}SO_2$, $r_{int}SO_2$, cerebral FTOE (cFTOE), liver FTOE (livFTOE), and intestinal FTOE (intFTOE) values. Means of rSO_2 and FTOE values were only used for further analyses if they were based on at least 30 minutes of available values.
The Mann-Whitney test was used to compare the 8-hour mean values of rSO_2 and FTOE between infants with suspected NEC and definite NEC and between infants with uncomplicated NEC and complicated NEC. Next, a receiver operating characteristics (ROC) curve was constructed of the rSO_2 values that were statistically significantly different between groups in the first 8 hours after onset of NEC symptoms to assess sensitivity and specificity and to determine potential cut-off values to predict the development of definite and complicated NEC within 48 hours after onset of NEC symptoms.
Finally, we determined the variability of the r_cSO_2, $r_{liv}SO_2$, and $r_{int}SO_2$ measurements separately during the first 48 hours after onset of NEC symptoms, by calculating each infant's daily

intraindividual variability, defined as the daily percentage of time that one-hour mean rSO_2 values were 15% or more below or above the infant's daily mean.[10]

To test whether there were differences between groups we used the chi-square test or Fisher exact test for categorical data and the Mann-Whitney test for continuous data. A *P* value of < .05 was considered statistically significant.

Since this study was of an exploratory nature, we refrained from performing extensive statistical analyses. Because clinical and radiographic symptoms and signs are evaluated every 8 to 12 hours in our neonatal intensive care unit, we decided to calculate 8-hour mean values of NIRS measurements in order to investigate the possibility of using rSO_2 and FTOE values in routine clinical care.

RESULTS

A total of 33 infants were included for final analysis (Figure 1). NIRS monitoring commenced after a median of 7 hours (range, 1-32) after onset of NEC symptoms. We were able to measure both $r_{liv}SO_2$ and $r_{int}SO_2$ in 24 infants. In seven infants we were unable to measure $r_{liv}SO_2$: in three infants due to shortage of equipment, and in four infants due to simultaneous inclusion in another multisite NIRS study in which renal rather than liver oxygen saturation was measured. In two infants we were unable to measure $r_{int}SO_2$: in one infant because we could not place the sensor due to the presence of an umbilical venous catheter taped to the infraumbilical skin, and in the other infant due to shortage of equipment.

We were able to calculate 8-hour mean r_cSO_2 values for 156, $r_{liv}SO_2$ for 115, and $r_{int}SO_2$ for 135 time periods out of the possible 198 (6 x 33 time periods). Median (range) time of available r_cSO_2, $r_{liv}SO_2$, and $r_{int}SO_2$ values every 8 hours was 450 (35-480), 370 (50-480), and 375 (30-480) minutes, respectively.

NIRS measurements in infants with suspected and definite NEC

Twenty infants developed Bell's stages 2 or 3 and thirteen infants ultimately did not have NEC (Bell's stage 1). Clinical diagnoses of the infants without NEC were sepsis (n=3), delayed passage of meconium (n=2), bloody stools of unknown cause (n=2), gastroenteritis (n=2), sigmoid volvulus (n=1), CPAP belly (n=1), and abdominal symptoms of unknown cause (n=2). Table 1 contains the patient characteristics of infants with suspected NEC and definite NEC. Infants with definite NEC underwent surgery significantly more often and had a higher mortality rate than infants with suspected NEC. Additionally, we found a trend towards a higher prevalence of anemia in preterm infants with suspected NEC than in infants with definite NEC.

In Table 2 we present the courses of rSO_2 and FTOE values. We found no significant differences between the two groups in the first 24 hours after onset of NEC symptoms. From 24 hours onwards, however, preterm infants with definite NEC had significantly higher median $r_{int}SO_2$ values in comparison to infants with suspected NEC. Furthermore, median

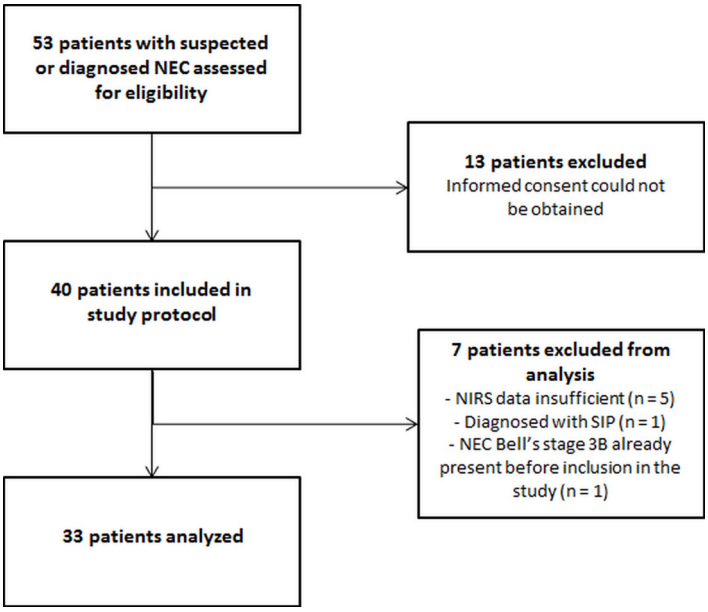

Figure 1. Flow diagram of the study.

intFTOE was significantly lower between 32 and 48 hours after onset of NEC symptoms in preterm infants with definite NEC in comparison to infants with suspected NEC.

NIRS measurements in infants with uncomplicated and complicated NEC

Ten out of twenty infants with definite NEC developed complicated NEC and the other ten infants developed NEC without complications. Of the infants with complicated NEC two were diagnosed with Bell's stage 3A. Both died as a consequence of NEC 5 and 35 days after onset of the symptoms. The other eight infants were found to have a perforation (Bell's stage 3B). Seven infants were operated on with a median time of 33 hours (range, 9-165) between onset of NEC symptoms and surgery. The other infant was too unstable clinically for surgery. Five infants were taken for surgery during the study period. NIRS monitoring in these infants was stopped after a median of 10 hours (range, 5-33) after onset of NEC symptoms. Of the infants with a perforation, five died as a consequence of NEC. Ischemic necrosis was confirmed by our pathologist using tissue macroscopy and microscopy in all infants with Bell's stage 3B.

In Table 3 we provide the patient characteristics of infants with an uncomplicated and a complicated course of NEC. Infants with complicated NEC were significantly younger, received inotropes more often, underwent surgery more often, and had a higher mortality rate than infants with uncomplicated NEC.

We present the courses of rSO_2 and FTOE values in Table 4. Preterm infants with complicated NEC had significantly lower median r_cSO_2 values throughout the entire study period and significantly higher cFTOE values from 8 hours onwards. Furthermore, we found lower

Table 1. Patient characteristics of infants with suspected and definite NEC.

	Suspected NEC (n = 13)	Definite NEC (n = 20)
Gestational age (weeks)	28.3 (27.0-31.7)	28.2 (25.0-35.9)
Birth weight (grams)	1190 (570-1690)	1333 (740-2400)
Male:Female	5:8	14:6§
PNA at first NIRS measurement (days)	13 (4-36)	10 (3-41)
Anemia (%)	8 (62)	6 (30)§
Thrombocytopenia (%)	1 (8)	5 (25)
Metabolic acidosis (%)	2 (17) (n = 12)	3 (16) (n = 19)
Mechanical ventilation (%)	3 (23)	6 (30)
Treated PDA before onset study (%)	2 (15)	5 (25)
PDA during study (%)	4 (31)	5 (25)
Hemodynamically significant	3	2
RBC transfusion (%)	4 (31)	7 (35)
Circulatory failure		
Fluid resuscitation (%)	5 (38)	12 (60)
Inotropes (%)	0 (-)	6 (30)§
Surgery (%)	0 (-)	9 (45)*
Death (%)	0 (-)	7 (35)*

Data are expressed as median (range) or as numbers unless specified otherwise.

Abbreviations: NEC - necrotizing enterocolitis; NIRS - near-infrared spectroscopy; PDA - patent ductus arteriosus; PNA - postnatal age; RBC - red blood cell.

Circulatory failure was defined as hemodynamic instability and scored by the need for volume expansion or the use of inotropes or both, from 1 hour before NEC onset until the first 48 hours after NEC onset, or until surgery, whichever came first.

Statistical differences between the two groups are marked by * (< .05) or § (< .10).

$r_{liv}SO_2$ and higher livFTOE values in preterm infants with complicated NEC than in infants with uncomplicated NEC in three time periods (0-8 hours, 24-32 hours, and 40-48 hours). Finally, $r_{int}SO_2$ was significantly lower and intFTOE higher between 8 and 16 hours and $r_{int}SO_2$ significantly higher and intFTOE lower between 24 and 32 hours after onset of NEC symptoms.

In Figure 2 we present the courses of the cerebral and splanchnic rSO_2 values in the first 48 hours after onset of NEC symptoms in infants with suspected NEC, uncomplicated NEC, and complicated NEC separately.

ROC curves

We generated ROC curves for r_cSO_2 and $r_{liv}SO_2$ to differentiate between infants with uncomplicated and complicated NEC, since only these values showed significant differences between the groups in the first 8 hours after onset of NEC symptoms. The area under the r_cSO_2 ROC curve was 0.88 (95% confidence interval (CI) 0.64-1.00, P = .047) and the area under the $r_{liv}SO_2$ curve was 1.00 (CI 1.00-1.00, P = .014). Taking a threshold value for r_cSO_2 of 71%, r_cSO_2 detected the presence of complications with a sensitivity of 1.00 (CI

Table 2. RSO_2 and FTOE values in the first 48 hours after onset of NEC symptoms in preterm infants with suspected and definite NEC.

	r_cSO_2		$r_{liv}SO_2$		$r_{int}SO_2$		cFTOE		livFTOE		intFTOE	
Hours	sNEC	dNEC	sNEC	dNEC	sNEC	dNEC	sNEC	dNEC	sNEC	dNEC	sNEC	dNEC
0-8	62% (7)	68% (10)	58% (5)	64% (9)	40% (8)	44% (8)	0.30 (7)	0.29 (8)	0.38 (5)	0.31 (8)	0.57 (8)	0.53 (6)
8-16	68% (12)	67% (14)	48% (9)	63% (11)	42% (11)	53% (13)	0.26 (12)	0.28 (14)	0.46 (9)	0.35 (11)	0.53 (11)	0.45 (13)
16-24	64% (13)	70% (15)	56% (8)	63% (12)	39% (13)	60% (11)	0.32 (13)	0.27 (14)	0.41 (8)	0.35 (12)	0.61 (13)	0.37§ (10)
24-32	65% (13)	72% (16)	59% (8)	55% (13)	40% (13)	56%* (10)	0.29 (13)	0.25 (16)	0.40 (8)	0.44 (13)	0.56 (13)	0.43§ (10)
32-40	65% (13)	72% (16)	49% (9)	59% (13)	38% (12)	55%* (13)	0.30 (13)	0.26 (16)	0.49 (9)	0.39 (13)	0.61 (12)	0.40* (13)
40-48	64% (12)	73% (15)	53% (7)	57% (12)	40% (10)	52%* (13)	0.30 (12)	0.25 (15)	0.45 (7)	0.41 (12)	0.57 (10)	0.42* (13)

Data are expressed as median values with the number of infants studied between brackets.
Statistical differences between the two groups are marked by * (< .05) or § (< .10).

0.46-1.00) and specificity of 0.80 (CI 0.30-0.99). Taking a threshold value for $r_{liv}SO_2$ of 59%, $r_{liv}SO_2$ detected the presence of complications with a sensitivity of 1.00 (CI 0.40-1.00) and specificity of 1.00 (CI 0.46-1.00).

Variability

In Table 5 we present the intraindividual variability. Variability measurements were neither significantly different within 24 hours after onset of NEC symptoms between infants with suspected and definite NEC, nor between infants with uncomplicated and complicated NEC. Between 24 and 48 hours, however, infants with definite NEC had significantly lower variability of $r_{int}SO_2$ measurements in comparison to infants with suspected NEC. Moreover, infants with complicated NEC had a significantly higher variability of r_cSO_2 and lower variability of $r_{int}SO_2$ measurements than infants with uncomplicated NEC.

DISCUSSION

Our study suggests that NIRS monitoring can be useful in preterm infants with definite NEC to differentiate in the first 8 hours after onset of symptoms between those infants who would develop complicated NEC and those who would not. The low oxygen saturation values and high oxygen extraction values of splanchnic and cerebral tissue are associated with the progression to a bowel perforation or death. Furthermore, we demonstrated that

Table 3. Patient characteristics of infants with uncomplicated and complicated NEC.

	Uncomplicated NEC (n = 10)	Complicated NEC (n = 10)
Gestational age (weeks)	30.9 (25.7-35.9)	27.2 (25.0-34.0)*
Birth weight (grams)	1518 (740-2400)	1035 (790-2280)[§]
Male:Female	6:4	8:2
PNA at first NIRS measurement (days)	10 (3-41)	10 (7-22)
Anemia (%)	3 (30)	3 (30)
Thrombocytopenia (%)	1 (10)	4 (40)
Metabolic acidosis (%)	1 (11) (n = 9)	2 (20)
Mechanical ventilation (%)	2 (20)	4 (40)
Treated PDA before onset study (%)	1 (10)	4 (40)
PDA during study (%)	1 (10)	4 (40)
Hemodynamically significant	0	2
RBC transfusion (%)	3 (30)	4 (40)
Circulatory failure		
Fluid resuscitation (%)	4 (40)	8 (80)
Inotropes (%)	0 (-)	6 (60)*
Surgery (%)	1 (10)	8 (80)*
Death (%)	0 (-)	7 (70)*

Data are expressed as median (range) or as numbers unless otherwise specified.

Abbreviations: NEC - necrotizing enterocolitis; NIRS - near-infrared spectroscopy; PDA - patent ductus arteriosus; PNA - postnatal age; RBC - red blood cell.

Circulatory failure was defined as hemodynamic instability and scored by the need for volume expansion or the use of inotropes or both, from 1 hour before NEC onset until the first 48 hours after NEC onset or until surgery took place, whichever came first. Differences between the two groups are marked by * (< .05) or [§] (< .10).

in the early stages of the disease with clinical signs pointing to NEC, NIRS monitoring did not help to differentiate between infants with definite NEC and infants who were diagnosed differently.

Distinguishing between NEC and other intestinal diseases is often difficult as symptoms are not specific. It is important, however, to be able to distinguish NEC from other abdominal illnesses as different diseases require different treatments. Early recognition of patients in need for surgery could also benefit the patient. To our knowledge no studies to date have investigated the possibility of NIRS monitoring to differentiate between preterm infants with suspected and preterm infants with definite NEC. Because hypoxia and/or necrosis of the bowel wall is present in preterm infants with NEC, we hypothesized that the rSO_2 values obtained in the splanchnic region would be lower and FTOE values higher than those measured in preterm infants who did not have NEC. Interestingly, in contrast to our hypothesis, we did not find significant differences between these two groups during the first 24 hours after onset of NEC symptoms. Perhaps the underlying conditions which were finally diagnosed in preterm infants with suspected NEC, such as volvulus and sepsis, had similar effects on the splanchnic rSO_2 and FTOE values obtained with NIRS as those observed

Table 4. RSO_2 and FTOE values in the first 48 hours after onset of NEC symptoms in preterm infants with uncomplicated and complicated NEC.

Hours	r_cSO_2		$r_{liv}SO_2$		$r_{int}SO_2$		cFTOE		livFTOE		intFTOE	
	unNEC	cNEC	unNEC	cNEC	unNEC	cNEC	unNEC	cNEC	unNEC	cNEC	unNEC	cNEC
0-8	83% (5)	65%* (5)	69% (5)	37%* (4)	77% (3)	43% (5)	0.13 (5)	0.42§ (3)	0.28 (5)	0.45* (3)	0.19 (3)	0.62§ (3)
8-16	81% (7)	55%* (7)	76% (7)	44% (4)	70% (6)	32%* (7)	0.17 (7)	0.38* (7)	0.22 (7)	0.53 (4)	0.27 (6)	0.54* (7)
16-24	81% (8)	54%* (7)	67% (8)	42% (4)	61% (7)	51% (4)	0.17 (8)	0.37* (6)	0.31 (8)	0.59 (4)	0.36 (7)	0.42 (3)
24-32	78% (9)	58%* (7)	60% (8)	31%* (5)	54% (8)	66%* (2)	0.21 (9)	0.35* (7)	0. 37 (8)	0.64* (5)	0.44 (8)	0.32* (2)
32-40	73% (10)	59%* (6)	59% (9)	53% (4)	48% (9)	59% (4)	0.22 (10)	0.35* (6)	0.39 (9)	0.44 (4)	0.48 (9)	0.38 (4)
40-48	75% (10)	55%* (5)	62% (9)	39%* (3)	47% (9)	55% (4)	0.24 (10)	0.38* (5)	0.37 (9)	0.59* (3)	0.53 (9)	0.40 (4)

Data are expressed as median values with the number of infants studied between brackets.

Statistical differences between the two groups are marked by * (< .05) or § (< .10).

in NEC. The higher prevalence of anemia in infants without NEC might also have contributed to the lower rSO_2 values in this group, since a low concentration of hemoglobin corresponds to lower oxygen saturation values.[11]

Although our results need to be confirmed in a larger patient population, this study suggested that NIRS did not serve the additional purpose of being able to distinguish between NEC and other intestinal diseases during the early stages of the disease .

Our second aim was to determine the value of splanchnic NIRS monitoring to predict a complicated course in preterm infants with definite NEC. In the first 24 hours after NEC onset, we demonstrated that both splanchnic and cerebral oxygen saturations were lower and that splanchnic and cerebral oxygen extractions were higher in preterm infants who developed complicated NEC.

We offer several explanations for these findings. First, blood flow to the splanchnic bed may be reduced due to the presence of ischemic/necrotic bowel. A second explanation relates to illness severity. This might have been so severe in those infants who developed

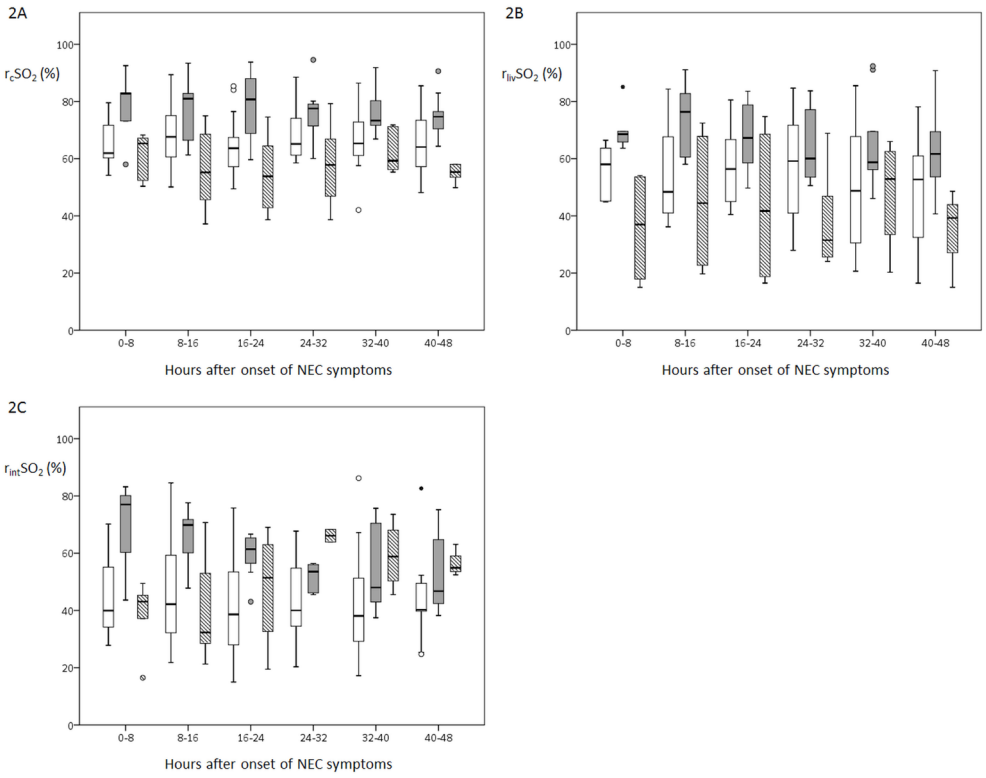

Figure 2. R_cSO_2, $r_{liv}SO_2$, and $r_{int}SO_2$ values in infants with suspected NEC, uncomplicated NEC, and complicated NEC. Data are shown in box and whisker plots. Dots and stars represent outliers.

- Suspected NEC
- Uncomplicated NEC
- Complicated NEC

Table 5. Intraindividual variability of preterm infants with suspected NEC versus preterm infants with definite NEC, and of preterm infants with uncomplicated NEC versus preterm infants with complicated NEC.

		Suspected NEC	Definite NEC	*P* value	Uncomplicated NEC	Complicated NEC	*P* value
0-24 hr	r_cSO_2 (%)	0 (0-39)	0 (0-14)	.106	0 (0-6)	0 (0-14)	.433
	$r_{liv}SO_2$ (%)	21 (0-44)	6 (0-42)	.421	12 (0-42)	6 (0-6)	.302
	$r_{int}SO_2$ (%)	16 (0-48)	15 (0-55)	.667	15 (0-55)	12 (0-36)	.999
24-48 hr	r_cSO_2 (%)	0 (0-14)	0 (0-33)	.951	0 (0-0)	4 (0-33)	.017*
	$r_{liv}SO_2$ (%)	13 (4-67)	13 (0-86)	.421	13 (4-86)	9 (0-25)	.393
	$r_{int}SO_2$ (%)	22 (0-78)	4 (0-25)	.022*	17 (0-25)	0 (0-4)	.022*

Data are expressed as median (range).

Abbreviations: NEC - necrotizing enterocolitis; r_cSO_2 - cerebral tissue oxygen saturation; $r_{liv}SO_2$ - liver tissue oxygen saturation; $r_{int}SO_2$ - infraumbilical tissue oxygen saturation. Intraindividual variability is defined as the daily percentage of time that one-hour mean rSO_2 values were 15% or more below or above the infant's daily mean.

* Indicates $P < .05$

complicated NEC that circulatory insufficiency ensued in the early stage of NEC. Perfusion to less essential organs, such as the intestine, will be affected first.[12] When insufficiency becomes more severe, however, the cerebral perfusion will also be compromised.[12] Indeed, cerebral oxygen saturation was lower and extraction was higher in preterm infants with complicated NEC than in preterm infants with uncomplicated NEC.

A third explanation would be that preterm infants with uncomplicated NEC might have had higher splanchnic oxygen saturation and lower oxygen extraction values due to a relatively increased intestinal blood flow compared to preterm infants with complicated NEC, caused by the inflammatory response seen in NEC. Increased blood flow velocities in the superior mesenteric artery and the celiac axis, the major contributors of blood flow to the intestinal tissue, have been shown in preterm infants with NEC compared to preterm infants without abdominal disease.[13,14] Moreover, McNeill *et al.* reported infraumbilical saturation values of 35% to 55% ten days after birth for relatively stable preterm infants between 29 to 33 weeks of gestation.[10] We found a higher median saturation level of 77% in the first 8 hours after onset of NEC symptoms in preterm infants with uncomplicated NEC.

Finally, the younger gestational age of infants with complicated NEC in comparison to infants with uncomplicated NEC might have contributed to the differences we found for splanchnic rSO_2 and FTOE values between these two groups.[10] This assumption, however, is based on a study performed in relatively healthy preterm infants who were fed normally.[10] The effect of NEC and its treatment on splanchnic oxygen saturation values makes an interpretation of the effect of gestational age on these values difficult, if not impossible.

Regarding variability measurements, we did not find any significant differences between preterm infants with suspected and definite NEC, and between infants with complicated

and uncomplicated NEC in the first 24 hours after onset of NEC symptoms. Although Cortez *et al.* suggested that loss of variability might be helpful to predict the onset of NEC our study suggests that these measurements might not be useful once NEC is suspected or diagnosed.[15]

In this study we have shown that values of $r_cSO_2 \leq 71\%$ and $r_{liv}SO_2 \leq 59\%$ predicted complicated NEC with a sensitivity of 1.00 and specificity of 0.80 and sensitivity of 1.00 and specificity of 1.00, respectively. These results suggest that monitoring cerebral and splanchnic rSO_2 might be helpful in clinical practice in predicting the course of NEC. However, we would like to stress the fact that these findings are based on measurements performed in a small sample size. Additionally, we did not control for potential confounders, such as gestational age and vasopressor medications. Our results, therefore, warrant further research in a larger patient population before we can be confident of its usefulness.

We acknowledge several limitations to our study. First of all, we included a relatively small sample size. Second, sensor replacement could unintentionally have caused variability in rSO_2 measurements. We do believe, however, that this phenomenon was distributed evenly over the various subgroups analyzed. Third, we did not include every preterm infant within 8 hours after onset of NEC symptoms and we were unable to measure the entire 48 hours in each infant which may have caused a sampling error. Finally, the splanchnic NIRS measurements might have been influenced by air, stools, movements of the gut within the abdominal cavity, and peristaltic movements.[3,16]

CONCLUSIONS

Our findings suggest that monitoring oxygen saturation and extraction at the cerebral and splanchnic region in preterm infants can help us to differentiate between complicated and uncomplicated NEC. However, we found no relevant added-value for NIRS in the diagnostic process of preterm infants with suspected NEC.

ACKNOWLEDGEMENTS

This study was part of the research program of the postgraduate school for Behavioural and Cognitive Neurosciences, University of Groningen. T.E. Schat and M.E. van der Laan were financially supported by a grant from the Junior Scientific Master Class of the University of Groningen.

We greatly acknowledge the help of Dr. T. van Wulfften Palthe for correcting the English manuscript.

REFERENCES

1. Roze E, Ta BD, van der Ree MH, et al. Functional impairments at school age of children with necrotizing enterocolitis or spontaneous intestinal perforation. *Pediatr Res*. 2011;70(6):619-625.
2. Hall NJ, Eaton S, Pierro A. Royal australasia of surgeons guest lecture. necrotizing enterocolitis: Prevention, treatment, and outcome. *J Pediatr Surg*. 2013;48(12):2359-2367.
3. Fortune PM, Wagstaff M, Petros AJ. Cerebro-splanchnic oxygenation ratio (CSOR) using near infrared spectroscopy may be able to predict splanchnic ischaemia in neonates. *Intensive Care Med*. 2001;27(8):1401-1407.
4. Zabaneh RN, Cleary JP, Lieber CA. Mesentric oxygen saturations in premature twins with and without necrotizing enterocolitis. *Pediatr Crit Care Med*. 2011;12(6):e404-6.
5. Stapleton GE, Eble BK, Dickerson HA, Andropoulos DB, Chang AC. Mesenteric oxygen desaturation in an infant with congenital heart disease and necrotizing enterocolitis. *Tex Heart Inst J*. 2007;34(4):442-444.
6. Naulaers G, Meyns B, Miserez M, et al. Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation. A validation study in piglets. *Neonatology*. 2007;92(2):120-126.
7. Watkins DJ, Besner GE. The role of the intestinal microcirculation in necrotizing enterocolitis. *Semin Pediatr Surg*. 2013;22(2):83-87.
8. Walsh MC, Kliegman RM. Necrotizing enterocolitis: Treatment based on staging criteria. *Pediatr Clin North Am*. 1986;33(1):179-201.
9. Schat TE, van der Laan ME, Schurink M, et al. Abdominal near-infrared spectroscopy in preterm infants: A comparison of splanchnic oxygen saturation measurements at two abdominal locations. *Early Hum Dev*. 2014;90(7):371-375.
10. McNeill S, Gatenby JC, McElroy S, Engelhardt B. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. *J Perinatol*. 2011;31(1):51-57.
11. van Hoften JC, Verhagen EA, Keating P, ter Horst HJ, Bos AF. Cerebral tissue oxygen saturation and extraction in preterm infants before and after blood transfusion. *Arch Dis Child Fetal Neonatal Ed*. 2010;95(5):F352-8.
12. Hanson SJ, Berens RJ, Havens PL, Kim MK, Hoffman GM. Effect of volume resuscitation on regional perfusion in dehydrated pediatric patients as measured by two-site near-infrared spectroscopy. *Pediatr Emerg Care*. 2009;25(3):150-153.
13. Kempley ST, Gamsu HR. Superior mesenteric artery blood flow velocity in necrotising enterocolitis. *Arch Dis Child*. 1992;67(7 Spec No):793-796.
14. Deeg KH, Rupprecht T, Schmid E. Doppler sonographic detection of increased flow velocities in the celiac trunk and superior mesenteric artery in infants with necrotizing enterocolitis. *Pediatr Radiol*. 1993;23(8):578-582.
15. Cortez J, Gupta M, Amaram A, Pizzino J, Sawhney M, Sood BG. Noninvasive evaluation of splanchnic tissue oxygenation using near-infrared spectroscopy in preterm neonates.*J Matern Fetal Neonatal Med*. 2011;24(4):574-582.
16. Teller J, Wolf M, Keel M, Bucher HU, Fanconi S, Baenziger O. Can near infrared spectroscopy of the liver monitor tissue oxygenation? *Eur J Pediatr*. 2000;159(7):549.

CHAPTER 7

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Trijntje E. Schat

Despite extensive research in the past few decades, predictive and preventive strategies for necrotizing enterocolitis (NEC) are lacking.[1] It is believed that early detection of and intervention in NEC might result in a reduction of short- and long-term complications. The ideal method to predict NEC would be non-invasive, portable, accurate, easy to use, cost-effective, and would have a high sensitivity and specificity for predicting NEC and its complications.[2] Currently, no apparatus meets these criteria. Near-infrared spectroscopy (NIRS), however, is non-invasive, portable, and easy to use. This tool provides insight in cerebral and splanchnic perfusion by measuring regional tissue oxygen saturation. Since it has been hypothesized that impaired intestinal perfusion is a critical step in the development of NEC,[3,4] NIRS might prove to be a valuable tool to predict this disease. The main aim of this thesis was therefore to investigate whether monitoring cerebral, liver, and intestinal oxygenation could be useful in infants who develop NEC. To this end, we formulated the following research questions:

(1) Is it feasible to study splanchnic oxygenation simultaneously in two abdominal regions in infants with suspected and definite NEC? Can liver and infraumbilical oxygen saturation values ($r_{liv}SO_2$ and $r_{int}SO_2$, respectively) substitute each other for the purpose of assessing splanchnic oxygenation? (Chapter 2)
(2) Can cerebral and splanchnic fractional tissue oxygen extraction (FTOE) values be used as markers for intestinal damage in infants with NEC? (Chapter 3)
(3) Do preterm infants with NEC show impaired cerebrovascular autoregulation (CAR) more often than infants without NEC? (Chapter 4)
(4) Can we differentiate high-risk infants who develop NEC from those who do not by monitoring cerebral and intestinal oxygenation as early as in the first days after birth? (Chapter 5)
(5) Can we, in an early stage of the disease, differentiate infants with definite NEC from infants with suspected NEC, and infants with complicated NEC from infants with uncomplicated NEC by monitoring cerebral, liver, and intestinal oxygenation? (Chapter 6)

MAIN FINDINGS

Is it feasible to study splanchnic oxygenation simultaneously in two abdominal regions in infants with NEC? Can $r_{liv}SO_2$ and $r_{int}SO_2$ values substitute each other for the purpose of assessing splanchnic oxygenation?

To address this question, we monitored $r_{liv}SO_2$ and $r_{int}SO_2$ simultaneously for 48 consecutive hours in preterm infants with suspected and definite NEC. We found that it was possible to monitor $r_{liv}SO_2$ and $r_{int}SO_2$ simultaneously 67% of the time. We did not encounter adverse skin effects or hindrance of routine clinical care. Additionally, we found a weak association between $r_{liv}SO_2$ and $r_{int}SO_2$ values and poor agreement between these values as assessed by a Bland-Altman plot.

Can cerebral and splanchnic FTOE values be used as markers for intestinal damage in infants with NEC?

To answer this question, we associated cerebral and splanchnic FTOE values with a marker for intestinal damage: intestinal fatty acid-binding protein in plasma (I-FABPp). I-FABP is primarily located in enterocytes of the small bowel and is secreted after compromised cell membrane integrity, such as occurs in intestinal ischemia and inflammation, including NEC.[5-8] During the first 16 hours after NEC onset, we found strong associations between cerebral and splanchnic FTOE values and I-FABPp levels in infants with NEC. From 16 hours after NEC onset, infants who developed complications as a result of NEC had both decreasing splanchnic FTOE values and I-FABPp levels, whilst infants with uncomplicated NEC showed increasing splanchnic FTOE values concurrent with decreasing I-FABPp levels.

Do preterm infants with NEC show impaired CAR more often than infants without NEC?

Nine infants with definite NEC (9/15, 60%) and five infant without NEC (5/13, 38%) had a statistically significant negative correlation between mean arterial blood pressure and cerebral FTOE, suggesting impaired CAR. The difference in prevalence of impaired CAR between these two groups was not statistically significant. Compared with the prevalence of impaired CAR in our control group (38%) and with the reported prevalence in infants without NEC in literature (40%),[9] infants with NEC in our study population had a high prevalence of impaired CAR.

Can we differentiate high-risk infants who develop NEC from those who do not by monitoring cerebral and intestinal oxygenation as early as in the first days after birth?

Infants with cerebral oxygen saturation (r_cSO_2) values < 70% (< 25th percentile) in the first two days after birth had a nine-fold higher risk of developing radiologically confirmed NEC than infants with r_cSO_2 values ≥ 70% (≥ 25th percentile). Intestinal oxygenation values obtained in the first days after birth were not predictive for NEC development. Two days prior to NEC development, we did find, however, higher intestinal FTOE values in infants who developed NEC compared with infants who did not develop NEC. Variability of r_cSO_2 and $r_{int}SO_2$ values in the first days after birth was not of additive value in predicting the onset of NEC.

Can we, in an early stage of the disease, differentiate infants with definite NEC from infants with suspected NEC, and infants with complicated NEC from infants with uncomplicated NEC by monitoring cerebral, liver, and intestinal oxygenation?

NIRS monitoring during the first 24 hours after onset of NEC symptoms in the cerebral as well as the splanchnic region did not distinguish infants with definite NEC from infants with suspected NEC. We did find, however, that liver rSO_2 values ≤ 59% in the first 8 hours after NEC onset predicted the development of complicated NEC in infants with definite NEC with a sensitivity and specificity of 1.00. Also, cerebral rSO_2 values ≤ 71% in the first 8 hours after NEC onset predicted complicated NEC with a sensitivity of 1.00 and a specificity of 0.80.

Variability of r_cSO_2, $r_{liv}SO_2$, and $r_{int}SO_2$ values had no additive predictive value for differentiating infants with definite NEC from infants with suspected NEC or for differentiating infants with complicated NEC from infants with uncomplicated NEC.

GENERAL DISCUSSION

Feasibility of measuring splanchnic oxygenation

Our first question was whether it was feasible to study splanchnic oxygenation in two abdominal regions simultaneously in preterm infants with NEC. For this purpose, we measured $r_{liv}SO_2$ and $r_{int}SO_2$ values concurrently for 48 consecutive hours in infants with suspected and definite NEC (Chapter 2). Median gestational age of the included infants was 28 weeks and median postnatal day of the first NIRS measurement was 9 days. We did not observe any problems concerning safety, such as adverse skin effects. Liver and intestinal rSO_2 could be measured simultaneously for 67% of the time; in five infants we were unable to monitor $r_{liv}SO_2$ and $r_{int}SO_2$ at the same time. In four infants this was due to shortage of equipment. In one infant, $r_{int}SO_2$ monitoring was not performed due to the lack of space for the sensor because of an umbilical venous catheter taped to the infraumbilical skin.

In contrast, we encountered more practical difficulties concerning intestinal oxygenation monitoring in the first two days after birth (Chapter 5). We measured $r_{int}SO_2$ in preterm infants at high risk of NEC who had a median gestational age of 28 weeks and a median birth weight of 955 grams. Intestinal oxygenation monitoring was possible in only seven out of the thirty infants (23%) in the first days after birth. In the remaining 23 infants adequate sensor placement was not possible due to the presence of an umbilicus venous catheter or lack of space in very low birth weight infants and infants small for gestational age. Theoretically, the placement of the umbilicus catheter could be easily adjusted. However, obviously, lack of space due to a very low birth weight or small for gestational age cannot be altered. Three other studies also measured intestinal oxygenation by means of NIRS in the infraumbilical region in the first days after birth.[10-12] McNeill *et al.* and Cortez *et al.* did not report practical difficulties.[10,11] However, compared with our study population, they included preterm infants with higher birth weights of median 1138 grams and mean 1640 grams.[10,11] Mintzer *et al.* included infants with a very low birth weight more similar to the birth weight of our population; they reported a median birth weight of 988 grams.[12] They also found that lack of space hindered adequate sensor placement. In these instances, they placed the sensor obliquely from the subumbilical region toward the left flank.[12] We did not replace the sensor, since we established that splanchnic oxygenation values differ when obtained in different abdominal regions (Chapter 2).

In conclusion, monitoring intestinal oxygenation at the infraumbilical region is feasible if enough space is available for adequate sensor placement. Since we did not apply a sensor in the liver region during the first days after birth, further studies are needed to determine the feasibility of monitoring the liver region during these first days.

Validity of measuring splanchnic oxygenation in infants with NEC

To investigate whether splanchnic oxygenation values can be used to assess intestinal damage in NEC, we associated liver and intestinal FTOE values with I-FABPp levels (Chapter 3). I-FABP is a protein that resides in epithelium cells of predominantly the small bowel.[6] In case of intestinal injury due to, amongst others, inflammation and ischemia, I-FABP is rapidly released into the circulation.[5-8] It was found that I-FABPp levels were associated with the development and severity of NEC.[7,8,13,14]

We found strong associations between liver and intestinal FTOE values on the one hand and I-FABPp levels on the other hand in preterm infants with NEC, suggesting that splanchnic FTOE values do indeed provide information about the degree of intestinal damage during NEC.

However, since we found poor agreement between $r_{liv}SO_2$ and $r_{int}SO_2$ values measured in infants with suspected and definite NEC (Chapter 2), we believe that site-specific factors might influence rSO_2 values. These site-specific factors may include, amongst others, differences in local intestinal and/or hepatic blood flow, interference by enteric contents, bowel movements within the abdominal cavity, and peristalsis.[10] These need particular attention in infants with NEC, since intestinal blood flow might be altered locally due to intestinal injury. Indeed, Zabaneh *et al.* found reduced cerebro-splanchnic oxygenation ratio (CSOR) values in certain, but not all, areas around the umbilicus in an infant with NEC.[15] They reported that the area in which they measured reduced CSOR values corresponded to the area of ischemic bowel, active inflammation, and adhesions as seen during surgery.[15] These results suggest that impaired oxygenation values due to NEC may only be detected when a NIRS sensor is applied to skin exactly overlapping intestinal injury. It would therefore be more informative to gather NIRS measurements of multiple abdominal regions (> 2) simultaneously. Currently, this has not yet been investigated. We speculate that measuring three or more abdominal locations at the same time in preterm infants might prove to be difficult for several reasons. First, as described previously, infants with a very low birth weight and infants small for gestational age might not have enough space on the abdominal wall to apply multiple sensors. Second, applying sensors in close proximity to each other might cause signal interference of the different sensors. The distance between light emitter and receivers of the neonatal SomaSensor is 40 mm at most. To avoid signal interference, the minimal distance between sensors should therefore be 40 mm. This undoubtedly limits the number of sensors that can be applied to the abdominal skin to monitor splanchnic oxygen saturation of different areas simultaneously. However, the fact that FTOE values obtained in the liver and intestinal region independently were strongly associated with I-FABPp levels still suggests that one location might be sufficient to assess the presence of intestinal damage in preterm infants with NEC. Further research is necessary to investigate the feasibility and necessity of monitoring splanchnic oxygen saturation of multiple abdominal locations simultaneously in infants with NEC.

Cerebral and splanchnic oxygenation and NEC

Table 1 summarizes our main findings. In the first two days after birth, we were able to differentiate high-risk infants who developed NEC from infants who did not using r_cSO_2 < 70%. In the week prior to NEC onset, intestinal FTOE values were significantly higher in infants who developed NEC compared with infants who did not (median 0.65 versus 0.44). Finally, in the first 8 hours after NEC onset, r_cSO_2 ≤ 71% and $r_{liv}SO_2$ ≤ 59% predicted the development of complicated NEC in infants with established NEC with a sensitivity of 1.00 and a specificity of 0.80 and a sensitivity and specificity of 1.00, respectively.

Pathophysiology of NEC

The occurrence of bowel ischemia is hypothesized to play a crucial role in the development of NEC.[3,4] The timing of this ischemic insult, however, remains unclear: it may be a primary inciting factor or it may be a secondary development as a result of intestinal injury and inflammation.[4] Several studies found indications of impaired splanchnic perfusion in the first days after birth in infants who developed NEC later on.[16-18] We therefore measured intestinal oxygenation in the first days after birth in high-risk preterm infants and assessed whether intestinal oxygenation values could enable us to discriminate between infants who developed NEC from infants who did not (Chapter 5). We also measured r_cSO_2 values since impaired intestinal perfusion might be the result of a compromised systemic circulation. We matched two control infants to each NEC case using the following criteria: gestational age, birth weight, and the presence of a hemodynamically significant patent ductus arteriosus. In the first two days after birth, we did not find differences in intestinal oxygenation values between infants who went on to develop NEC and infants who did not. However, infants who developed NEC had lower r_cSO_2 values compared with infants who did not develop NEC. These findings suggest that, in those infants, the systemic circulation is compromised during the first two days after birth.

Numerous prenatal and postnatal factors have been associated with the development of NEC, including but not limited to maternal infections,[19,20] placental insufficiency resulting in growth-restricted newborns,[21-23] resuscitation at birth,[24] mechanical ventilation in the first days of life,[24,25] and a hemodynamically significant patent ductus arteriosus.[25,26] Associations were found between lower r_cSO_2 values and ascending intrauterine infection,[27] resuscitation at birth,[28,29] and hemodynamically significant patent ductus arteriosus.[30] One or more of the aforementioned factors might have contributed to the lower cerebral oxygenation values we found in preterm infants who developed NEC.

Decreased blood flow to cerebral tissue almost always occurs when compensatory flow redistribution away from the less essential organs, such as the splanchnic tissue, has failed.[31] We therefore speculate that splanchnic perfusion is also affected at birth. In the first two days after birth, we found a median $r_{int}SO_2$ of 44% in infants who went on to develop NEC compared with 50% in infants who did not develop NEC; this difference was not statistically significant. Our small sample size might have hindered us from finding significant differences

Table 1. Key Findings.

		Key findings		
		First two days after birth	One week before NEC onset	First eight hours after NEC onset
NEC versus no NEC in high-risk infants (Chapter 5)	Cerebral	Infants with r_cSO_2 < 70% developed NEC 9 times more often than infants with r_cSO_2 ≥ 70%	Not significant	NA
	Liver	NI	NI	NA
	Intestinal	Not significant	intFTOE was significantly higher in infants who developed NEC compared with infants who did not (median 0.65 versus 0.44).	NA
Complicated NEC versus uncomplicated NEC in high-risk infants	Cerebral	Not significant	Not significant	NA
	Liver	NI	NI	NA
	Intestinal	Not significant	Not significant	NA
Definite NEC versus suspected NEC in infants with abdominal symptoms (Chapter 6)	Cerebral	NA	NA	Not significant
	Liver	NA	NA	Not significant
	Intestinal	NA	NA	Not significant
Complicated NEC versus uncomplicated NEC in infants with established NEC (Chapter 6)	Cerebral	NA	NA	r_cSO_2 ≤ 71% predicted complicated NEC with a sensitivity of 1.00 and a specificity of 0.80.
	Liver	NA	NA	$r_{liv}SO_2$ ≤ 59% detected the presence of complicated NEC with a sensitivity of 1.00 and a specificity of 1.00.
	Intestinal	NA	NA	Not significant

intFTOE - intestinal fractional tissue oxygen extraction; NEC - necrotizing enterocolitis; NA - not applicable; NI - not investigated; r_cSO_2 - cerebral oxygen saturation; $r_{liv}SO_2$ - liver oxygen saturation.

in intestinal oxygenation values between infants who developed NEC and infants who did not. In line of the proposed hypothesis, it could be suggested that the first insult to intestinal tissue can already occur before or shortly after birth, predisposing an infant to developing NEC later on.

Two days prior to NEC onset on median postnatal day eight, we found significantly higher intestinal FTOE values in preterm infants who developed NEC compared with infants who did not develop NEC (Chapter 5). This finding suggests that impaired splanchnic perfusion is present before the clinical onset of NEC. Postnatal factors that might have negatively influenced splanchnic perfusion are anemia,[32-34] feeding practices, especially during red blood cell transfusion,[35-37] and a hemodynamically significant patent ductus arteriosus.[38,39] These factors have also been found to increase the risk of developing NEC.[25,26,40,41] We speculate that a combination of multiple prenatal and/or postnatal factors eventually contribute to intestinal ischemia and injury and the subsequent development of NEC.

Once NEC had developed, we clearly observed distinct courses of simultaneously measured I-FABP levels in plasma and splanchnic FTOE values for infants with complicated NEC compared with infants with uncomplicated NEC in the first 48 hours after NEC onset (Chapter 3). Infants with an uncomplicated and complicated course showed decreasing I-FABPp levels during the development of NEC. This might be the result of either recovery of intestinal tissue or extension of intestinal injury. Based on the course of splanchnic FTOE values we ventured to discriminate between these two hypothesized mechanisms. Infants with complicated NEC showed high splanchnic FTOE values in the first 16 hours after NEC onset that gradually decreased in the 32 hours that followed. We speculate that intestinal perfusion is compromised in infants with complicated NEC as a result of decreased splanchnic metabolism due to the presence of necrotic bowel. In infants with uncomplicated NEC splanchnic FTOE values were low in the first 16 hours after NEC onset and gradually increased afterwards. Possibly, hyperemia is present which allows the intestinal tissue to recover. Impaired splanchnic perfusion seems, therefore, to be a major determinant in the development of complications once NEC has been clinically diagnosed.

In conclusion, our results suggest that the first insult or first 'hit' on intestinal tissue that predisposes an infant to NEC development might occur as early as before birth. One or multiple insults afterwards, for example due to anemia and/or the presence of a hemodynamically significant patent ductus arteriosus might eventually contribute to impaired intestinal perfusion and the subsequent development of NEC. The responsible factors might differ between individual patients. After NEC has developed, sustained intestinal hypoperfusion induces the development of complications, i.e. bowel perforation or death. Based on our results, impaired intestinal perfusion might be both an inciting factor as well as a secondary event in NEC development.

Prognostic value of cerebral and splanchnic NIRS monitoring in predicting and diagnosing NEC

It was suggested that values of CSOR might be more helpful than splanchnic oxygenation monitoring alone in predicting and diagnosing bowel ischemia.[42] However, to be able to use CSOR, cerebral perfusion must be maintained during periods of splanchnic hypoperfusion due to the presence of CAR.[42] We established that CAR was not present in a considerable proportion (60%) of infants with NEC. Using cerebral oxygenation as reference for splanchnic oxygenation therefore seems unreliable. We therefore investigated whether both cerebral and splanchnic oxygenation monitoring could independently enable us to predict and diagnose NEC and its complications timely.

Due to a possible link between intestinal perfusion and NEC,[3,4] studies have focused on determining the usefulness of splanchnic oxygenation values, specifically values obtained in the infraumbilical region, for predicting and detecting NEC early on.[15,17,18,42,43] Our results, however, suggest that rSO_2 values obtained in the liver region might be more appropriate for this purpose. In contrast to the non-significant findings for $r_{int}SO_2$ values, all infants with definite NEC who had $r_{liv}SO_2$ values ≤ 59% in the first 8 hours after NEC, developed complicated NEC. Monitoring the liver region for the purpose of predicting and diagnosing NEC might have some benefits over monitoring the infraumbilical region. First of all, as opposed to intestinal tissue, the liver is a solid and non-moving organ; it was found that the precision of NIRS depends on tissue homogeneity.[44] Second, applying the infraumbilical sensor is associated with practical difficulties, especially in the first days after birth. We do not expect these difficulties to be present when monitoring the liver region: there is usually enough space, also during the first days after birth, and no catheters are taped to the skin just below the right costal margin. Larger patient studies are warranted to examine the additive value of monitoring $r_{liv}SO_2$ in the first days after birth and whether liver oxygenation values might be more suitable than intestinal oxygenation values for the purpose of predicting and diagnosing NEC and its complications timely.

The findings of this thesis suggest that cerebral oxygenation monitoring might also be of additive value in predicting and diagnosing NEC. We found that infants with r_cSO_2 values < 70% in the first days after birth developed NEC nine times more often later on than infants with r_cSO_2 values ≥ 70%. Moreover, we found that all infants who would develop complicated NEC had r_cSO_2 values ≤ 71% in the first 8 hours after clinical NEC onset, compared with 20% of infants who would not develop complicated NEC. Monitoring cerebral rSO_2 values offers several important benefits over monitoring splanchnic rSO_2 values. First of all, cerebral oxygenation values are more robust with little variability compared with splanchnic rSO_2 values which are more inconsistent, possibly due to the influence of enteric contents, peristalsis, and movements of intestinal tissue within the abdominal cavity.[10] Second, cerebral NIRS monitoring is already used in clinical practice as a monitoring device and a large multicenter randomized trial is currently investigating whether cerebral oxygenation values can be implemented in treatment guidelines.[45] Splanchnic NIRS monitoring on the

other hand is presently used for research purposes only. Finally, we found that monitoring the infraumbilical region is associated with practical difficulties in the first days after birth, thereby limiting the application in clinical practice. Based on the findings presented in this thesis, we cannot draw a definite conclusion concerning the superiority of cerebral or splanchnic NIRS monitoring in infants at risk of NEC or infants with NEC. It is to be determined whether cerebral and/or splanchnic NIRS monitoring is preferred for this purpose.

Cortez *et al.* suggested that variability measurements of splanchnic rSO_2 values might contribute to predict the onset of NEC.[11] They described the course of intestinal rSO_2 values in two infants who developed NEC. One infant had extremely low $r_{int}SO_2$ values with loss of variability followed by very high $r_{int}SO_2$ values, whilst the other infant illustrated the opposite: very high $r_{int}SO_2$ values followed by extremely low $r_{int}SO_2$ values with loss of variability.[11] We calculated variability measurements based on the definition set by McNeill *et al*.[10] We did not find variability measurements of cerebral and splanchnic rSO_2 values to be of additive value in predicting and diagnosing NEC and its complications. Interpretation of individual courses of oxygenation values will possibly be more informative than the use of median or mean values of group of infants. Further research is necessary to confirm this hypothesis.

FUTURE PERSPECTIVES

To date, no tool or test is available that can accurately predict the onset and development of NEC and its complications. In our opinion, the results of this thesis suggest that cerebral and splanchnic NIRS monitoring are of additive value for this purpose. It is, however, yet to be determined whether both the cerebral and splanchnic region need to be monitored by NIRS or if one of these locations suffices. Moreover, since our results suggest that splanchnic oxygenation values differ when obtained in different abdominal regions, further research should also focus on investigating the predictive value of monitoring one or multiple abdominal regions in infants at high risk of developing NEC or in infants with established NEC.

Since the cut-off values we presented in this thesis are based on rather small sample sizes, larger multicenter trials are needed to provide more definite cut-off values. Additionally, the clinical relevance of these cut-off values is yet to be determined. With the current knowledge and available treatment options, we suggest that infants with low cerebral and/or splanchnic rSO_2 values in the first days after birth and in the first 8 hours after NEC onset should be monitored more intensively. Deterioration might thus be noticed earlier, which may lead to a more timely intervention. Moreover, further research is warranted to investigate treatment options that can improve intestinal perfusion, since impaired splanchnic perfusion seems to play an important role in the development of NEC and complicated NEC. Several studies found increased intestinal blood flow after administration of potential therapeutic targets in animal models.[46-49] These possible therapeutic options should be studied further.

Finally, the use of cerebral and splanchnic NIRS monitoring in infants with NEC should not be limited to predict NEC and complicated NEC. NIRS might have an additional application

in helping determine readiness to accept full feeds after NEC treatment and the effect of surgery on CAR in infants with NEC. These possible applications should be studied as well to improve neonatal and neurological outcome.

REFERENCES

1. Lin PW, Nasr TR, Stoll BJ. Necrotizing enterocolitis: Recent scientific advances in pathophysiology and prevention. *Semin Perinatol*. 2008;32(2):70-82.
2. Moore JE. Newer monitoring techniques to determine the risk of necrotizing enterocolitis. *Clin Perinatol*. 2013;40(1):125-134.
3. Nowicki PT. Ischemia and necrotizing enterocolitis: Where, when, and how. *Semin Pediatr Surg*. 2005;14(3):152-158.
4. Watkins DJ, Besner GE. The role of the intestinal microcirculation in necrotizing enterocolitis. *Semin Pediatr Surg*. 2013;22(2):83-87.
5. Kanda T, Fujii H, Tani T, et al. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology*. 1996;110(2):339-343.
6. Lieberman JM, Sacchettini J, Marks C, Marks WH. Human intestinal fatty acid binding protein: Report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery*. 1997;121(3):335-342.
7. Edelson MB, Sonnino RE, Bagwell CE, Lieberman JM, Marks WH, Rozycki HJ. Plasma intestinal fatty acid binding protein in neonates with necrotizing enterocolitis: A pilot study. *J Pediatr Surg*. 1999;34(10):1453-1457.
8. Guthmann F, Borchers T, Wolfrum C, Wustrack T, Bartholomaus S, Spener F. Plasma concentration of intestinal- and liver-FABP in neonates suffering from necrotizing enterocolitis and in healthy preterm neonates. *Mol Cell Biochem*. 2002;239(1-2):227-234.
9. Verhagen EA, Hummel LA, Bos AF, Kooi EM. Near-infrared spectroscopy to detect absence of cerebrovascular autoregulation in preterm infants. *Clin Neurophysiol*. 2014;125(1):47-52.
10. McNeill S, Gatenby JC, McElroy S, Engelhardt B. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. *J Perinatol*. 2011;31(1):51-57.
11. Cortez J, Gupta M, Amaram A, Pizzino J, Sawhney M, Sood BG. Noninvasive evaluation of splanchnic tissue oxygenation using near-infrared spectroscopy in preterm neonates. *J Matern Fetal Neonatal Med*. 2011;24(4):574-582.
12. Mintzer JP, Parvez B, Chelala M, Alpan G, LaGamma EF. Quiescent variability of cerebral, renal, and splanchnic regional tissue oxygenation in very low birth weight neonates. *J Neonatal Perinatal Med*. 2014;7(3):199-206.
13. Aydemir C, Dilli D, Oguz SS, et al. Serum intestinal fatty acid binding protein level for early diagnosis and prediction of severity of necrotizing enterocolitis. *Early Hum Dev*. 2011;87(10):659-661.
14. Heida FH, Hulscher JBF, Schurink M, et al. Intestinal fatty acid-binding protein levels in necrotizing enterocolitis correlate with extent of necrotic bowel: results from a multicenter study. *J Pediatr Surg*. 2014.
15. Zabaneh RN, Cleary JP, Lieber CA. Mesentric oxygen saturations in premature twins with and without necrotizing enterocolitis. *Pediatr Crit Care Med*. 2011;12(6):e404-6.
16. Murdoch EM, Sinha AK, Shanmugalingam ST, Smith GC, Kempley ST. Doppler flow velocimetry in the superior mesenteric artery on the first day of life in preterm infants and the risk of neonatal necrotizing enterocolitis. *Pediatrics*. 2006;118(5):1999-2003.
17. Gay AN, Lazar DA, Stoll B, et al. Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. *J Pediatr Surg*. 2011;46(6):1034-1040.

18. Patel AK, Lazar DA, Burrin DG, et al. Abdominal near-infrared spectroscopy measurements are lower in preterm infants at risk for necrotizing enterocolitis. *Pediatr Crit Care Med*. 2014;15(8):735-741.
19. Been JV, Lievense S, Zimmermann LJ, Kramer BW, Wolfs TG. Chorioamnionitis as a risk factor for necrotizing enterocolitis: A systematic review and meta-analysis. *J Pediatr*. 2013;162(2):236-42. e2.
20. Ducey J, Owen A, Coombs R, Cohen M. Vasculitis as part of the fetal response to acute chorioamnionitis likely plays a role in the development of necrotizing enterocolitis and spontaneous intestinal perforation in premature neonates. *Eur J Pediatr Surg*. 2014.
21. Bhatt AB, Tank PD, Barmade KB, Damania KR. Abnormal doppler flow velocimetry in the growth restricted foetus as a predictor for necrotising enterocolitis. *J Postgrad Med*. 2002;48(3):182-5; discussion 185.
22. Manogura AC, Turan O, Kush ML, et al. Predictors of necrotizing enterocolitis in preterm growth-restricted neonates. *Am J Obstet Gynecol*. 2008;198(6):638.e1-638.e5.
23. Kamoji VM, Dorling JS, Manktelow B, Draper ES, Field DJ. Antenatal umbilical doppler abnormalities: An independent risk factor for early onset neonatal necrotizing enterocolitis in premature infants. *Acta Paediatr*. 2008;97(3):327-331.
24. Gregory KE. Clinical predictors of necrotizing enterocolitis in premature infants. *Nurs Res*. 2008;57(4):260-270.
25. Gagliardi L, Bellu R, Cardilli V, De Curtis M, Network Neonatale Lombardo. Necrotising enterocolitis in very low birth weight infants in italy: Incidence and non-nutritional risk factors. *J Pediatr Gastroenterol Nutr*. 2008;47(2):206-210.
26. Cassady G, Crouse DT, Kirklin JW, et al. A randomized, controlled trial of very early prophylactic ligation of the ductus arteriosus in babies who weighed 1000 g or less at birth. *N Engl J Med*. 1989;320(23):1511-1516.
27. Roescher AM, Timmer A, van der Laan ME, et al. In preterm infants, ascending intrauterine infection is associated with lower cerebral tissue oxygen saturation and higher oxygen extraction. *Pediatr Res*. 2015.
28. Fuchs H, Lindner W, Buschko A, Trischberger T, Schmid M, Hummler HD. Cerebral oxygenation in very low birth weight infants supported with sustained lung inflations after birth. *Pediatr Res*. 2011;70(2):176-180.
29. Binder C, Urlesberger B, Avian A, Pocivalnik M, Muller W, Pichler G. Cerebral and peripheral regional oxygen saturation during postnatal transition in preterm neonates. *J Pediatr*. 2013;163(2):394-399.
30. Lemmers PM, Toet MC, van Bel F. Impact of patent ductus arteriosus and subsequent therapy with indomethacin on cerebral oxygenation in preterm infants. *Pediatrics*. 2008;121(1):142-147.
31. Hanson SJ, Berens RJ, Havens PL, Kim MK, Hoffman GM. Effect of volume resuscitation on regional perfusion in dehydrated pediatric patients as measured by two-site near-infrared spectroscopy. *Pediatr Emerg Care*. 2009;25(3):150-153.
32. Wardle SP, Weindling AM. Peripheral fractional oxygen extraction and other measures of tissue oxygenation to guide blood transfusions in preterm infants. *Semin Perinatol*. 2001;25(2):60-64.
33. Dani C, Pratesi S, Fontanelli G, Barp J, Bertini G. Blood transfusions increase cerebral, splanchnic, and renal oxygenation in anemic preterm infants. *Transfusion*. 2010;50(6):1220-1226.
34. Sandal G, Oguz SS, Erdeve O, Akar M, Uras N, Dilmen U. Assessment of red blood cell transfusion and transfusion duration on cerebral and mesenteric oxygenation using near-infrared spectroscopy in preterm infants with symptomatic anemia. *Transfusion*. 2014;54(4):1100-1105.

35. Dani C, Pratesi S, Barp J, et al. Near-infrared spectroscopy measurements of splanchnic tissue oxygenation during continuous versus intermittent feeding method in preterm infants. *J Pediatr Gastroenterol Nutr*. 2013;56(6):652-656.
36. Corvaglia L, Martini S, Battistini B, Rucci P, Aceti A, Faldella G. Bolus vs. continuous feeding: Effects on splanchnic and cerebral tissue oxygenation in healthy preterm infants. *Pediatr Res*. 2014;76(1):81-85.
37. Marin T, Josephson CD, Kosmetatos N, Higgins M, Moore JE. Feeding preterm infants during red blood cell transfusion is associated with a decline in postprandial mesenteric oxygenation. *J Pediatr*. 2014;165(3):464-71.e1.
38. Meier SD, Eble BK, Stapleton GE, Morales DL, Chang AC, Andropoulos DB. Mesenteric oxyhemoglobin desaturation improves with patent ductus arteriosus ligation. *J Perinatol*. 2006;26(9):562-564.
39. Petrova A, Bhatt M, Mehta R. Regional tissue oxygenation in preterm born infants in association with echocardiographically significant patent ductus arteriosus. *J Perinatol*. 2011;31(7):460-464.
40. Christensen RD, Lambert DK, Henry E, et al. Is "transfusion-associated necrotizing enterocolitis" an authentic pathogenic entity? *Transfusion*. 2010;50(5):1106-1112.
41. Singh R, Visintainer PF, Frantz ID,3rd, et al. Association of necrotizing enterocolitis with anemia and packed red blood cell transfusions in preterm infants. *J Perinatol*. 2011;31(3):176-182.
42. Fortune PM, Wagstaff M, Petros AJ. Cerebro-splanchnic oxygenation ratio (CSOR) using near infrared spectroscopy may be able to predict splanchnic ischaemia in neonates. *Intensive Care Med*. 2001;27(8):1401-1407.
43. Stapleton GE, Eble BK, Dickerson HA, Andropoulos DB, Chang AC. Mesenteric oxygen desaturation in an infant with congenital heart disease and necrotizing enterocolitis. *Tex Heart Inst J*. 2007;34(4):442-444.
44. Arri SJ, Muehlemann T, Biallas M, Bucher HU, Wolf M. Precision of cerebral oxygenation and hemoglobin concentration measurements in neonates measured by near-infrared spectroscopy. *J Biomed Opt*. 2011;16(4):047005.
45. Hyttel-Sorensen S, Pellicer A, Alderliesten T, et al. Cerebral near infrared spectroscopy oximetry in extremely preterm infants: Phase II randomised clinical trial. *BMJ*. 2015;350:g7635.
46. Gill RS, Manouchehri N, Lee TF, et al. Cyclosporine treatment improves mesenteric perfusion and attenuates necrotizing enterocolitis (NEC)-like intestinal injury in asphyxiated newborn piglets during reoxygenation. *Intensive Care Med*. 2012;38(3):482-490.
47. Maki AC, Matheson PJ, Shepherd JA, Garrison RN, Downard CD. Intestinal microcirculatory flow alterations in necrotizing enterocolitis are improved by direct peritoneal resuscitation. *Am Surg*. 2012;78(7):803-807.
48. Walker SK, Matheson PJ, Schreiner MT, Smith JW, Garrison RN, Downard CD. Intraperitoneal 1.5% delflex improves intestinal blood flow in necrotizing enterocolitis. *J Surg Res*. 2013;184(1):358-364.
49. Matheson PJ, Walker SK, Maki AC, Shaheen SP, Neal Garrison R, Downard CD. Oral relaxin maintains intestinal blood flow in a rat model of NEC. *J Pediatr Surg*. 2014;49(6):961-4; discussion 964-5.

CHAPTER 8

SUMMARY IN ENGLISH

SAMENVATTING IN HET NEDERLANDS

ABBREVIATIONS

DANKWOORD

ABOUT THE AUTHOR

SUMMARY IN ENGLISH

General introduction

The main aim of the research presented in this thesis was to investigate whether monitoring cerebral, liver, and intestinal oxygenation could enable us to predict the onset of necrotizing enterocolitis (NEC) and its complications. NEC is currently the most common and deadliest gastrointestinal disease in preterm infants. Prevalence of NEC ranges between 7 and 11%. Although neonatal care has improved drastically in the last few decades, the short- and long-term consequences of NEC have not improved concurrently. Overall, mortality rates range between 9 and 40%, being the highest in infants with low birth weights and infants with the most advanced stages of NEC. Other complications include intestinal stricture, abdominal abcess, cholestasis, and short bowel syndrome. Moreover, neurodevelopmental impairments frequently occur later on, specifically in infants who are treated surgically for NEC.

The pathophysiology of NEC remains largely unknown. Several factors have been identified that play a putative role in the development of NEC. Results of animal and human studies suggest that impaired intestinal perfusion might be one of these factors. Being able to detect this altered perfusion may give the clinician an early warning about the onset and progression of this disease. A non-invasive, bedside tool that reflects splanchnic perfusion might therefore prove to be helpful.

Splanchnic perfusion can be assessed indirectly by measuring splanchnic oxygen saturation and extraction by means of near-infrared spectroscopy (NIRS). NIRS is based on the fact that light in the near-infrared range (wavelengths between 700 and 1000 nm) can be effectively transmitted through biological tissue over longer distances. Oxygenated and deoxygenated hemoglobin will absorb one portion of this near-infrared light. A second part of this light will be scattered and a third part will be reflected. Since oxygenated and deoxygenated hemoglobin have distinct absorption spectra, NIRS can differentiate between the two. By measuring the spectral absorption of these two chromophores, the ratio of oxygenated hemoglobin to total hemoglobin can be calculated. This measurement represents the oxygen uptake in tissue and is referred to as regional tissue oxygen saturation (rSO_2). Approximately 75 to 80% of this value forms a representation of the saturation of venous blood, 5% forms the capillary compartment and the remaining percentage is from arterial blood.

When the transcutaneous arterial oxygen saturation (SpO_2) is measured simultaneously, the fractional tissue oxygen extraction (FTOE) can be calculated by the following equation: FTOE = (SpO_2 - rSO_2)/ SpO_2. FTOE is thought to reflect the balance between tissue oxygen delivery and tissue oxygen consumption and might therefore be an early indicator of impaired tissue perfusion. High FTOE values can indicate two possibilities: (1) increased oxygen extraction due to increased metabolism at the tissue level, or (2) increased oxygen extraction due to decreased blood flow to the tissue that is being measured. For the purpose of this thesis, we used the INVOS 5100C spectrometer (Covidien, Mansfield, MA, USA) with neonatal SomaSensors (Covidien).

The results of several studies suggested that NIRS might indeed be valuable to predict the onset and development of NEC; lower intestinal oxygen and higher extraction values were found in infants who went on to develop NEC and in infants with NEC. However, since impaired intestinal perfusion might be the result of a compromised systemic circulation, it would also be interesting to measure cerebral oxygenation values.

The main aim of this thesis was whether monitoring cerebral, liver, and intestinal oxygenation could be useful in infants who develop NEC. It led to the following research questions:

(1) Is it feasible to study splanchnic oxygenation simultaneously in two abdominal regions in infants with suspected and definite NEC? Can liver and infraumbilical oxygen saturation values ($r_{liv}SO_2$ and $r_{int}SO_2$, respectively) substitute each other for the purpose of assessing splanchnic oxygenation? (Chapter 2)
(2) Can cerebral and splanchnic FTOE values be used as markers for intestinal damage in infants with NEC? (Chapter 3)
(3) Do preterm infants with NEC show impaired cerebrovascular autoregulation (CAR) more often than infants without NEC? (Chapter 4)
(4) Can we differentiate high-risk infants who develop NEC from those who do not by monitoring cerebral and intestinal oxygenation as early as in the first days after birth? (Chapter 5)
(5) Can we, in an early stage of the disease, differentiate infants with definite NEC from infants with suspected NEC, and infants with complicated NEC from infants with uncomplicated NEC by monitoring cerebral, liver, and intestinal oxygenation? (Chapter 6)

Feasibility of splanchnic NIRS monitoring

We found that it was feasible to monitor splanchnic oxygenation in the liver and infraumbilical region simultaneously in infants with suspected and definite NEC for 48 consecutive hours. We did not encounter practical difficulties or problems concerning safety. We were able to obtain $r_{liv}SO_2$ and $r_{int}SO_2$ values concurrently during two-thirds of the study period. Additionally, we found a weak association between $r_{liv}SO_2$ and $r_{int}SO_2$ values, and a poor agreement between these two values as assessed by a Bland-Altman plot.

Validity of measuring cerebral and splanchnic oxygenation in preterm infants with NEC

For this purpose, we associated cerebral and splanchnic FTOE values with levels of intestinal fatty acid-binding protein in plasma (I-FABPp), a marker for intestinal damage, in preterm infants with definite NEC. I-FABP is primarily located in enterocytes of the small bowel and is secreted after compromised cell membrane integrity, such as occurs in intestinal ischemia and inflammation, including NEC. We found strong associations in the first 16 hours after NEC onset between cerebral and splanchnic FTOE values on the one hand and I-FABPp levels on the other hand. Additionally, we observed distinct courses for combined splanchnic FTOE and I-FABPp levels in infants with uncomplicated NEC and complicated NEC. We defined

complicated NEC as the infant developing a bowel perforation requiring surgery (Bell's stage 3B), or death. From 16 hours after NEC onset, we found that both splanchnic FTOE values and I-FABPp levels decreased in preterm infants with complicated NEC. In preterm infants with uncomplicated NEC splanchnic FTOE values gradually increased while I-FABPp levels decreased. We observed this increase and decrease of splanchnic FTOE values particularly for the intestinal region and not for the liver.

CAR in preterm infants with NEC

60% of preterm infants with definite NEC were found to have a statistically significant negative association between mean arterial blood pressure and cerebral FTOE, indicating impaired CAR. This is high compared with the percentage of impaired CAR in infants who do not have NEC (38%), although this difference was not statistically significant.

Cerebral and intestinal oxygenation values in infants at high risk of developing NEC

We found that infants with cerebral oxygen saturation (r_cSO_2) values < 70% in the first two days after birth developed radiologically confirmed NEC nine times more often than infants with r_cSO_2 values ≥ 70%. Intestinal oxygenation values obtained in the first two days after birth, on the contrary, were not predictive for NEC development. However, we were only able to measure $r_{int}SO_2$ in seven out of the thirty infants due to practical difficulties, especially in infants with very low birth weights and who are small for gestational age. These infants lacked space for adequate sensor placement. Two days prior to NEC onset, however, we did find higher intestinal FTOE values in infants who went on to develop NEC compared with infants who did not. We also calculated variability measurement of cerebral and intestinal rSO_2 values based on a definition first used by McNeill *et al.* We did not find significant differences in cerebral and intestinal variability measurements between infants who went on to develop NEC and infants who did not; neither in the first two days after birth nor two days prior to NEC onset.

Cerebral and splanchnic oxygenation values in infants with NEC

We were unable to differentiate infants with definite NEC from infants with suspected NEC based on cerebral and splanchnic oxygenation values obtained in the first 24 hours after onset of NEC symptoms. It was, however, possible to distinguish infants with complicated NEC from infants with uncomplicated NEC using NIRS: all infants with $r_{liv}SO_2$ values ≤ 59% in the first 8 hours after NEC onset developed complicated NEC. Additionally, all infants who would develop complicated NEC had r_cSO_2 values ≤ 71% in the first 8 hours after NEC onset, compared with 20% of infants who would develop uncomplicated NEC. Variability of r_cSO_2, $r_{liv}SO_2$, and $r_{int}SO_2$ values could not be used for differentiating between infants with definite and suspected NEC and between infants with complicated and uncomplicated NEC.

Implications and future perspectives

Our study has four important implications. First, monitoring splanchnic oxygenation in two abdominal locations in preterm infants with abdominal signs and symptoms is feasible and safe. However, practical difficulties limit the usefulness of monitoring intestinal oxygenation in preterm infants in the first days after birth. In particular, infants who are small for gestational age and infants who have a very low birth weight, lack space for adequate sensor placement. The feasibility of monitoring liver oxygenation in the first days after birth needs to be investigated further.

Second, splanchnic oxygenation values provide information about the degree of intestinal damage in infants with NEC. In our study, splanchnic FTOE values were strongly associated with I-FABPp levels. However, since we also found a poor agreement between $r_{liv}SO_2$ and $r_{int}SO_2$ values, site-specific factors, such as differences in local intestinal and/or hepatic blood flow, interference by enteric contents, bowel movements within the abdominal cavity, and peristalsis, may influence rSO_2 values. Infants with NEC may have an altered blood flow locally due to intestinal injury. Measuring splanchnic oxygenation in three or more abdominal regions might therefore lead to earlier detection of intestinal damage. The feasibility and necessity of this option, however, needs to be further elucidated.

Third, our findings imply that impaired intestinal perfusion is both an inciting factor as well as a secondary event in the development of NEC. Additionally, compromised systemic circulation may be present in the first two days after birth in infants who develop NEC later on. The latter finding suggests that the first insult leading to intestinal underperfusion and a subsequent increased risk of developing NEC may occur before, during, or shortly after birth. Multiple insults afterwards due to, amongst others, anemia and/or the presence of a hemodynamically significant patent ductus arteriosus, may eventually lead to impaired intestinal perfusion and intestinal injury, as seen in NEC. After NEC became clinically evident, we observed sustained intestinal hypoperfusion in infants who developed complications, i.e. bowel perforation and/or death. Our findings suggest that new treatment options of NEC should focus on improving intestinal perfusion.

Fourth, both cerebral and splanchnic NIRS monitoring seem useful in predicting NEC and its complications timely. The cut-off values presented in this thesis, however, need to be confirmed in larger multicenter trials. Furthermore, we may also benefit from investigating additional applications of NIRS monitoring in infants with NEC, such as to determine readiness to accept full feeds after NEC treatment and the effect of surgery on CAR, with the ultimate goal of improving neonatal and neurological outcome.

SAMENVATTING IN HET NEDERLANDS

Introductie

Het hoofddoel van dit proefschrift was om vast te stellen of het meten van de cerebrale, lever en intestinale zuurstofvoorziening ons in staat zou kunnen stellen om het ontstaan van necrotiserende enterocolitis (NEC) en de bijbehorende complicaties te voorspellen. NEC is de meest voorkomende gastrointestinale aandoening bij prematuur geboren kinderen met een prevalentie die varieert van 7 tot 11%. Ondanks een verbetering van de neonatale zorg in de afgelopen decennia, zijn zowel de korte als de lange termijn gevolgen van NEC niet verbeterd. De mortaliteit van NEC varieert tussen de 9 en 40%, waarbij het hoogste sterftecijfer gezien wordt bij prematuur geboren kinderen met een extreem laag geboortegewicht en bij pasgeborenen die het meest gevorderde stadium van NEC ontwikkelen. Andere complicaties van NEC zijn vernauwingen in het maag-darmkanaal, abdominale abcessen, cholestase en het kortedarmsyndroom. Bovendien hebben prematuren die NEC gehad hebben een grotere kans op neurologische ontwikkelingsproblemen, vooral wanneer NEC ook chirurgisch behandeld is.

De pathofysiologie van NEC is nog grotendeels onbekend. Onderzoek heeft aangetoond dat een verminderde intestinale doorbloeding wellicht bijdraagt aan het ontwikkelen van NEC. Het vroegtijdig opsporen van deze veranderde doorbloeding zou mogelijk kunnen leiden tot het eerder vaststellen van NEC en/of het eerder opmerken van verergering van NEC naar een vergevorderd stadium. Een methode die op een niet-invasieve manier de splanchnische doorbloeding meet, zou daarom een waardevolle toevoeging kunnen zijn in de diagnostiek van NEC.

Met behulp van nabij-infrarood licht spectroscopie (NIRS) is het mogelijk om de splanchnische doorbloeding indirect te kunnen schatten door het meten van de splanchnische zuurstofvoorziening. NIRS zendt nabij-infrarood licht uit dat het golflengtegebied omspant tussen 700 en 1000 nm. Dit nabij-infrarood licht onderscheidt zich van licht met andere golflengtes, omdat het op een effectieve manier een langere afstand door biologisch weefsel kan afleggen. Een deel van het nabij-infrarode licht wordt door het zuurstofgebonden hemoglobine en zuurstofvrije hemoglobine geabsorbeerd. Daarnaast wordt een deel verspreid door het weefsel en een deel wordt gereflecteerd. Aangezien het zuurstofgebonden en zuurstofvrije hemoglobine elk zijn eigen karakteristiek absorptiespectrum heeft, kan NIRS een onderscheid maken tussen deze beide moleculen. De ratio tussen het zuurstofgebonden hemoglobine en het totale hemoglobine bepaalt het regionale weefsel zuurstofgehalte (rSO_2). Ongeveer 75 tot 80% van deze waarde bestaat uit het zuurstofgehalte gemeten in het veneuze bloed, 5% uit het capillaire compartiment en het resterende percentage wordt gevormd door het arteriële bloed.

Wanneer tegelijkertijd de transcutane arteriële zuurstofsaturatie (SpO_2) gemeten wordt, kan de fractionele weefsel zuurstofextractie (FTOE) berekend worden met behulp van de volgende formule: $FTOE = (SpO_2 - rSO_2)/ SpO_2$. Omdat FTOE beschouwd wordt als de balans tussen de aanvoer van zuurstof aan weefsel en het verbruik van zuurstof in weefsel, wordt

deze waarde gebruikt om een indruk te krijgen van de weefseldoorbloeding. Hoge FTOE waarden kunnen wijzen op twee mogelijkheden: (1) verhoogde zuurstofextractie door een toename in het metabolisme, of (2) verhoogde zuurstofextractie door een verminderde bloeddoorstroming. Alle studies in dit proefschrift werden uitgevoerd met de INVOS 5100C spectrometer (Covidien, Mansfield, MA, USA) en de neonatale SomaSensoren (Covidien).
Uit meerdere onderzoeken is gebleken dat NIRS een mogelijk toegevoegde waarde heeft in het voorspellen van het ontstaan van NEC en de verergering van NEC naar een vergevorderd stadium; NEC was geassocieerd met lagere intestinale zuurstofsaturatie waarden en hogere intestinale zuurstofextractie waarden. Aangezien een verminderde intestinale doorbloeding ook een resultaat kan zijn van een verminderde systemische doorbloeding, zou het tevens interessant zijn om naast de intestinale doorbloeding de cerebrale zuurstofvoorziening te meten.

Het hoofddoel van dit proefschrift was daarom vast te stellen of het meten van de cerebrale, lever en intestinale zuurstofvoorziening van toegevoegde waarde zou kunnen zijn in het diagnostisch proces bij prematuur geboren kinderen die NEC gaan ontwikkelen of die NEC ontwikkeld hebben. Deze vraag leidde tot de volgende onderzoeksvragen:

(1) Is het haalbaar en uitvoerbaar om splanchnische zuurstofvoorziening gelijktijdig te meten op twee abdominale locaties bij prematuur geboren kinderen met NEC? Kunnen zuurstofsaturatie waarden gemeten in het gebied van de lever en de regio onder de navel elkaar vervangen om een indruk te krijgen van de splanchnische zuurstofvoorziening? (Hoofdstuk 2)
(2) Kunnen cerebrale en splanchnische FTOE waarden gebruikt worden als ‘markers’ voor intestinale schade bij prematuur geboren kinderen met NEC? (Hoofdstuk 3)
(3) Is de cerebrovasculaire autoregulatie (CAR) vaker gestoord bij prematuur geboren kinderen met NEC dan bij prematuur geboren kinderen zonder NEC? (Hoofdstuk 4)
(4) Is het mogelijk om hoog-risico prematuren die later NEC ontwikkelen te onderscheiden van hoog-risico prematuren die later geen NEC ontwikkelen door het meten van de cerebrale en intestinale zuurstofvoorziening in de eerste dagen na de geboorte? (Hoofdstuk 5)
(5) Is het mogelijk om prematuur geboren kinderen met bewezen NEC (Bell’s stadium ≥ 2) vroegtijdig te onderscheiden van prematuren die verdacht worden van NEC (Bell’s stadium 1), en prematuren met gecompliceerde NEC van prematuren met ongecompliceerde NEC door het meten van de cerebrale, lever en intestinale zuurstofvoorziening? (Hoofdstuk 6)

Haalbaarheid en uitvoerbaarheid van het meten van de splanchnische zuurstofvoorziening

Uit ons onderzoek bleek dat het mogelijk was om de splanchnische zuurstofvoorziening simultaan te meten gedurende 48 uur in het gebied van de lever en de regio onder de navel bij prematuren die verdacht werden van NEC of gediagnosticeerd waren met NEC.

We ondervonden geen praktische moeilijkheden of problemen met betrekking tot de veiligheid. We waren in staat om lever zuurstofsaturatie ($r_{liv}SO_2$) waarden en intestinale zuurstofsaturatie ($r_{int}SO_2$) waarden gelijktijdig te verkrijgen gedurende tweederde van de studieperiode. Tevens vonden we een zwakke associatie tussen $r_{liv}SO_2$ en $r_{int}SO_2$ waarden en een slechte overeenkomst tussen deze twee waarden, vastgesteld aan de hand van een Bland-Altman plot.

Validiteit van het meten van cerebrale en splanchnische zuurstofvoorziening bij prematuur geboren kinderen met NEC

Teneinde een antwoord op deze vraag te kunnen geven, hebben we cerebrale en splanchnische FTOE waarden gecorreleerd met de concentratie van het intestinale-type vetzuur bindend eiwit in plasma (I-FABPp) bij prematuren met bewezen NEC. I-FABPp is een maat voor intestinale schade en bevindt zich voornamelijk in enterocyten van de dunne darm. Het wordt uitgescheiden nadat de integriteit van de celmembraan van de enterocyt is gecompromitteerd, zoals bij intestinale ischemie en inflammatie, waaronder NEC.
In de eerste 16 uren na het ontstaan van NEC, vonden wij sterke associaties tussen cerebrale en splanchnische FTOE waarden aan de ene kant en concentraties van I-FABPp aan de andere kant. Vanaf 16 uren na het ontstaan van NEC, zagen we een verschillend beloop van splanchnische FTOE waarden en I-FABPp concentraties bij prematuren met gecompliceerde NEC ten opzichte van prematuren met ongecompliceerde NEC. Hierbij hebben wij gecompliceerde NEC gedefinieerd als het ontwikkelen van een darmperforatie waarvoor een chirurgische behandeling vereist is (Bell's stadium 3B) of overlijden ten gevolge van NEC. Bij prematuren met gecompliceerde NEC daalden zowel de splanchnische FTOE waarden als de I-FABPp concentraties. Bij prematuren met ongecompliceerde NEC daarentegen, stegen de splanchnische FTOE waarden en daalden de I-FABPp concentraties. Deze stijging en daling van splanchnische FTOE waarden zagen we voornamelijk bij waarden die we verkregen bij de regio onder de navel en minder bij de waarden gemeten ter plaatse van de lever.

CAR bij prematuur geboren kinderen met NEC

60% van de prematuren met bewezen NEC hadden een statistisch significante negatieve associatie tussen de gemiddelde arteriële bloeddruk en de cerebrale FTOE waarden, oftewel een bloeddruk afhankelijke hersendoorbloeding, indicatief voor een gestoorde CAR. Deze waarde is hoog in vergelijking met het percentage van gestoorde CAR bij prematuren zonder NEC (38%), alhoewel het verschil niet statistisch significant was.

Cerebrale en intestinale zuurstofvoorziening bij prematuren met een hoog risico op het ontwikkelen van NEC

Prematuur geboren kinderen met cerebrale zuurstofsaturatie (r_cSO_2) waarden < 70% in de eerste twee dagen na de geboorte ontwikkelden bewezen NEC negen keer vaker dan prematuren met r_cSO_2 waarden ≥ 70%. De intestinale zuurstofvoorziening gemeten met

NIRS in de eerste twee dagen na de geboorte was niet significant verschillend tussen prematuren die later wel NEC ontwikkelden en prematuren die later niet NEC ontwikkelden. Niettemin, $r_{int}SO_2$ kon gemeten worden in slechts zeven van de dertig prematuren. Dit had te maken met praktische moeilijkheden, voornamelijk bij prematuren met een erg laag geboortegewicht en prematuren met een te kleine lengte en/of een te klein gewicht voor de zwangerschapsduur. Bij deze prematuren was er niet genoeg plaats om de sensor op een juiste manier te kunnen bevestigen. Twee dagen vóór het ontstaan van NEC, vonden we wel hogere intestinale FTOE waarden bij prematuren die NEC gingen ontwikkelen vergeleken met prematuren die geen NEC ontwikkelden. Als laatste hebben we de variabiliteit van de cerebrale en intestinale rSO_2 waarden berekend, gebaseerd op een berekening van McNeill *et al*. We vonden geen verschil in variabiliteit tussen prematuren die wel NEC ontwikkelden en prematuren die geen NEC ontwikkelden; noch voor de eerste dagen na de geboorte, noch twee dagen voor het ontstaan van NEC.

Cerebrale en splanchnische zuurstofvoorziening bij prematuren met NEC

Uit ons onderzoek is gebleken dat het niet mogelijk is om op basis van de cerebrale en splanchnische zuurstofvoorziening, gemeten in de eerste 24 uur na het ontstaan van symptomen die mogelijk passen bij NEC, een onderscheid te maken tussen prematuren met een uiteindelijk bewezen NEC en prematuren met alleen een verdenking op NEC, die uiteindelijk geen NEC bleken te hebben. Het was wel mogelijk om met NIRS de prematuren met een gecompliceerde NEC vroegtijdig te onderscheiden van prematuren met een ongecompliceerde NEC: alle prematuren met $r_{liv}SO_2$ waarden ≤ 59% in de eerste 8 uur na het ontstaan van een bewezen NEC ontwikkelden uiteindelijk complicaties. Tevens vonden we dat alle prematuren die een gecompliceerde NEC ontwikkelden, r_cSO_2 waarden ≤ 71% hadden in de eerste 8 uur na het ontstaan van een bewezen NEC in vergelijking met 20% van prematuren die een ongecompliceerde NEC ontwikkelden. Variabiliteit van r_cSO_2, $r_{liv}SO_2$ en $r_{int}SO_2$ waarden kon niet gebruikt worden om te differentiëren tussen prematuren met een bewezen NEC en prematuren met alleen een verdenking op NEC en ook niet tussen prematuren met gecompliceerde NEC en ongecompliceerde NEC.

Implicaties en vooruitzichten

De resultaten die in dit proefschrift gepresenteerd zijn, hebben vier belangrijke implicaties. Ten eerste hebben we aangetoond dat het haalbaar en veilig is om de splanchnische zuurstofvoorziening gelijktijdig te meten op twee verschillende abdominale locaties bij prematuren met abdominale symptomen. Echter, praktische moeilijkheden beperken de mogelijkheid om de intestinale zuurstofvoorziening te meten bij prematuur geboren kinderen in de eerste dagen na de geboorte. Voornamelijk prematuren met een erg laag geboortegewicht en/of prematuren met een te kleine lengte en/of een te klein gewicht voor de zwangerschapsduur hebben onvoldoende ruimte om de SomaSensor correct te

bevestigen. De haalbaarheid van het meten van de lever zuurstofvoorziening in de eerste dagen na de geboorte dient verder onderzocht te worden.

Ten tweede hebben we aangetoond dat splanchnische FTOE waarden informatie verschaffen over de mate van intestinale schade bij prematuren met NEC: splanchnische FTOE waarden waren sterk gecorreleerd met I-FABPp concentraties. Aangezien we tevens een slechte overeenkomst vonden tussen $r_{liv}SO_2$ en $r_{int}SO_2$ waarden, is het mogelijk dat locatiespecifieke factoren, zoals verschillen in lokale intestinale en/of hepatische bloedstroom, storing door darminhoud, darmbewegingen in de abdominale holte en peristaltiek, de rSO_2 waarden beïnvloeden. Prematuren met NEC hebben mogelijkerwijs een verminderde lokale doorbloeding door schade aan het intestinale weefsel. Het meten van de splanchnische zuurstofvoorziening in drie of meer abdominale locaties zou daarom potentieel tot een eerdere detectie kunnen leiden van intestinale schade. De haalbaarheid en zinvolheid van deze optie moet echter nader onderzocht worden.

Ten derde impliceren onze bevindingen dat een verminderde intestinale doorbloeding zowel een rol speelt in het ontstaan van NEC als een secundair effect is in de ontwikkeling van NEC. Bovendien hebben we aangetoond dat ook een verminderde systemische zuurstofvoorziening aanwezig kan zijn in de eerste twee dagen na de geboorte bij prematuur geboren kinderen die later NEC ontwikkelen. Deze laatste bevinding suggereert dat de eerste vatbaarheid voor het later ontwikkelen van NEC al kan ontstaan voor, gedurende of kort na de geboorte. Het gezamenlijk effect met meerdere predisponerende factoren nadien, zoals anemie en/of de aanwezigheid van een hemodynamisch significante patente ductus arteriosus, zouden kunnen leiden tot een verminderde intestinale doorbloeding en de ontwikkeling van intestinale schade, zoals gezien wordt in NEC.

Nadat NEC had geleid tot klinische symptomen, zagen we dat een verminderde intestinale doorbloeding nog steeds aanwezig was bij prematuren met gecompliceerde NEC. Daarentegen lieten prematuren met ongecompliceerde NEC herstel van de intestinale doorbloeding zien. Op basis van onze bevindingen zouden we willen aanbevelen dat nieuwe therapeutische strategieën bij NEC zich richten op de verbetering van de intestinale doorbloeding.

Ten vierde, zowel het meten van de cerebrale als splanchnische weefsel zuurstofsaturatie en –extractie lijken van toegevoegde waarde te zijn in het voorspellen van NEC en de geassocieerde complicaties. De grenswaarden die in dit proefschrift genoemd worden, zouden echter in grotere trials bevestigd moeten worden.

Het gebruik van NIRS bij prematuren met NEC zou niet beperkt moeten blijven tot de toepassingen beschreven in dit proefschrift. Zo zou NIRS een bijdragende rol kunnen spelen in het bepalen wanneer prematuren na het doormaken van NEC weer volledig enteraal gevoed zouden kunnen worden, of het onderzoeken wat het effect van een chirurgische behandeling van NEC is op CAR. Het uiteindelijke doel van al deze onderzoeken zou steeds dezelfde moeten zijn, namelijk het verbeteren van de neonatale en neurologische uitkomst van prematuren met NEC.

ABBREVIATIONS

BW	birth weight
CAR	cerebrovascular autoregulation
CBF	cerebral blood flow
CI	confidence interval
CPAP	continuous positive airway pressure
CRP	C-reactive protein
CSOR	cerebro-splanchnic oxygenation ratio
GA	gestational age
Hb	hemoglobin
HFOV	high-frequency oscillatory ventilation
FTOE	fractional tissue oxygen extraction
cFTOE	cerebral fractional tissue oxygen extraction
livFTOE	liver fractional tissue oxygen extraction
intFTOE	intestinal fractional tissue oxygen extraction
I-FABPp	intestinal fatty acid-binding protein in plasma
INVOS	In Vivo Optical Spectroscopy
MABP	mean arterial blood pressure
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
NIMV	nasal intermittent mandatory ventilation
NIRS	near-infrared spectroscopy
pCO_2	partial pressure of carbon dioxide
PDA	patent ductus arteriosus
PNA	postnatal age
PPROM	preterm premature rupture of membranes
RBC	red blood cell
ROC	receiver operating characteristics
rSO_2	regional tissue oxygen saturation
r_cSO_2	cerebral oxygen saturation
$r_{liv}SO_2$	liver oxygen saturation
$r_{int}SO_2$	intestinal/infraumbilical oxygen saturation
SD	standard deviation
SIMV	synchronized intermittent mandatory ventilation
SiPAP	synchronised intermittent positive airway pressure
SIPPV	synchronous positive pressure ventilation
SpO_2	transcutaneous arterial oxygen saturation

DANKWOORD

Met het schrijven van dit dankwoord leg ik de laatste hand aan mijn proefschrift. De afgelopen 2,5 jaar zijn een intensieve periode geweest waarin velen mij hebben gesteund, geënthousiasmeerd, en waar nodig een hart onder de riem hebben gestoken. In het bijzonder wil ik de volgende mensen bedanken.

Allereerst wil ik alle ouders en hun kinderen bedanken die hebben deelgenomen aan de onderzoeken. Wij mochten deel uitmaken van een bijzondere, maar ook heftige periode in jullie leven. In mijn ogen heeft dit het onderzoek nog waardevoller gemaakt.

Prof. dr. Bos, beste Arie, zonder jou was het mij niet gelukt om dit proefschrift tot een succes te maken. Ik kon jou altijd bereiken voor advies, ook al was het rond middernacht. Bedankt voor jouw inspirerende samenwerking en begeleiding van de afgelopen jaren.

Dr. Kooi, beste Elisabeth, wat ben ik blij dat jij mijn co-promotor bent! Onze bijna nooit geplande overlegjes waren niet alleen productief, maar ook erg gezellig. Jouw bevlogenheid en enthousiasme werken aanstekelijk en ik ben je dankbaar dat je mij hebt aangespoord om het MD/PhD-traject te gaan doen. Ook al heb ik ervoor gekozen om geen carrière in de kindergeneeskunde na te streven, toch hoop ik dat we elkaar in de toekomst niet uit het oog verliezen.

Dr. Hulscher, beste Jan, ik heb veel gehad aan jouw inhoudelijke kritiek en ideeën en je suggesties om het onderzoek en de artikelen beter te maken. Bedankt voor je nooit aflatende enthousiasme.

De leden van de Leescommissie, **Prof. dr. E. Heineman**, **Prof. dr. F. van Bel, en Prof. dr. I. Reiss**, wil ik allen hartelijk bedanken voor het kritisch lezen en beoordelen van mijn proefschrift.

Graag wil ik alle medewerkers van de **afdeling Neonatologie en afdeling Kinderchirurgie** in het Universitair Medisch Centrum Groningen bedanken voor hun hulp bij alle grote en kleine klussen. Mijn speciale dank gaat uit naar **Annelies Olthuis** voor al haar hulp bij ons onderzoek. Verder wil ik alle **verpleegkundigen, neonatologen, kinderchirurgen en fellows** bedanken voor hun prettige samenwerking rondom het NIRS onderzoek.

Co-auteurs, geen van de artikelen had bestaan zonder jullie hulp. Ik wil daarom alle co-auteurs bedanken, voor zover al niet genoemd. Bedankt voor jullie inzet, taalkundige, inhoudelijke en statistische toevoegingen.

Mede MD/PhD-studenten van de afgelopen jaren, promoveren doe je niet alleen. Bedankt voor alle gezellige kletsmomentjes met eindeloos veel kopjes thee. Anne, Annemiek, Janyte,

Jozien, Marrit, Michelle, Mirthe, Sara, Sietske en Tjitske, bedankt! In het bijzonder wil ik Anne, Michelle, Mirthe en Sara noemen. Wat ben ik blij dat ik samen met jullie het NIRS onderzoek mocht doen. Ik wil jullie bedanken voor al jullie hulp, inhoudelijk en praktisch, en relativeer momentjes. Ook wil ik **alle collega's in het Triade** en **alle studenten** die mee hebben geholpen aan de onderzoeken bedanken.

Prof. dr. A.E.J. Dubois, Dr. T. van Wulfften Palthe en P.H. de Zeeuw, bedankt voor alle tijd en moeite die jullie hebben gestoken in het corrigeren van de artikelen in dit proefschrift op het Engels.

Prof. H. Marike Boezen, bedankt voor uw statistische hulp.

De **Junior Scientific Masterclass** wil ik bedanken voor de mogelijkheid die ik heb gekregen om dit onderzoek in de vorm van een MD/PhD-traject uit te voeren.

Fred de Geus en Frans Erich, dank voor de ICT-ondersteuning. Het was soms even wat gepuzzel, maar het was uiteindelijk de moeite waard. De Raspberry Pi's hangen en werken!

Mijn **paranimfen Marianne en Tineke**. Lieve Marianne, leaf suske, wat ben ik trots op jou! Nog twee maanden en dan sta je hier zelf om je bul in ontvangst te nemen om daarna je eerste stappen als 'echte' dokter te zetten. Ik heb bewondering voor je doorzettingsvermogen en je wilskracht. Ik kan alleen maar zeggen dat ik het een eer vind dat je vandaag naast mij wilt staan. Lieve Tineke, lieve 'grote zus', want dat ben je voor mij. Als ik even iets niet weet of ergens niet uitkom, kan ik je altijd om advies en raad vragen. Bedankt dat je op deze speciale dag naast mij wilt staan.

Lieve **vrienden, familie en schoonfamilie**, bedankt dat jullie er altijd voor mij zijn, ondanks mijn drukke agenda.

Leave **heit en mem**, ik kom woorden tekort om uit te drukken hoeveel jullie voor mij betekenen. Jullie staan altijd voor mij klaar en zonder jullie eindeloze steun en liefde was ik niet gekomen waar ik nu ben.

Mijn aller-, allerliefste **Jacob**, jij bent mijn steun en toeverlaat. Jouw onvoorwaardelijke liefde en luisterend oor zijn voor mij van onschatbare waarde. Jij was degene die mij een bakje thee met een stukje chocolade op precies de juiste momenten bracht en mij nog steeds helpt met het maken van figuren voor in de artikelen. Straks breekt voor ons weer een hele nieuwe spannende fase in ons leven aan. Ik kan me niet een ander persoon voorstellen waarmee ik dit geluk wil delen. Ik hou van je!

ABOUT THE AUTHOR

Nynke (Trijntje Eelkje) Dijkstra-Schat was born on February 2, 1988 in Dokkum, Friesland, the Netherlands. She graduated from the gymnasium of Dockinga College with honour in 2006, a secondary school in Dokkum. During that same year, she started medical school at the University of Groningen. In 2012 she started her scientific clerkship as part of her final year of medicine with Prof. dr. A.F. Bos en Dr. E.M.W. Kooi at the division of Neonatology, University Medical Center Groningen. During this clerkship her enthusiasm concerning research with preterm infants grew, which eventually led to the application for the challenging MD-PhD trajectory, a programme that allows students to obtain a PhD degree in an additional two years next to their regular study. She was accepted for this trajectory in November 2012. She first finished her final clinical rotations at the Departement of Pediatrics at University Medical Center Groningen and Antonius Ziekenhuis Sneek. She graduated from medical school with honour in February 2013. This same month she started her two years of clinical research at University Medical Center Groningen. The results of this research are presented in this thesis. She was awarded the Best Plenary Presentation Award during the Neonatal Fellow Days in Rotterdam, 2013. Furthermore, she has presented her research at several international and national conferences. As of March 2014, she is working as a general practitioner in training in a Health Care Center in Groningen.